Estrès, reproducció i senescència en l'èxit invasor: l'estudi de cas de *Carpobrotus edulis*

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Memòria presentada per Erola Fenollosa Romaní per optar al grau de Doctora per la Universitat de Barcelona.

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Doctoranda:	Director i tutor de Tesi:
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Als meus pares, la Montserrat i l’Artur.

És una mala amiga que ve de sota a terra i es fica a la cuina. No té vergonya i em pren les coses bones.

És una bona amiga, transporta les llavors, recicla les deixalles, espanta els cargols i fa dibuixos a la sorra.

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Abstract

Invasive species constitute one of the main threats to global biodiversity. Especially those areas hosting a huge number of native species, such as the Mediterranean basin, are sensitive to these change drivers. *Carpobrotus edulis* is a widespread clonal invasive species that has become invasive at the Mediterranean basin reducing native biodiversity by strongly impacting the physicochemical soil properties. We aimed to understand *C. edulis* invasive success through an ecophysiological approach by understanding the relationships between its stress tolerance, reproduction capacity and the role of senescence in its fitness, which together define the species' life history strategy. Our results showed that *C. edulis* is adapted to the Mediterranean basin environmental conditions showing a high physiological plasticity in response to drought and chilling, the most sensitive periods along the year in the Mediterranean area. The species stress tolerance was crucial to invade Europe as revealed by the projected species distribution model and the increased chilling tolerance found in *C. edulis* invasive individuals. *C. edulis* is a monocarpic clonal species at the ramet level but polycarpic at the genet level, which constitutes and adaptive senescence process to growth facilitation to the next generations. High reproductive effort, seed production and seed longevity contribute to the formation of a persistent seed bank for this species increasing its impact and persistence potential. Seed trait variability across the invaded range generates differential soil seed bank dynamics that may be considered for improving management policies. This variability at different scales may contribute to species success by resource optimization, perturbation resilience and allowing adaptation. Overall, the *C. edulis* invasion at the Mediterranean basin results from a positive feedback of expansion and impacts through the interaction of species stress tolerance, reproductive effort and adaptive senescence. We conclude that the invasion of *C. edulis* in Europe has been possible not only by its ideal traits such as clonality, strong reproductive effort, the formation of a persistent soil seed bank, short life cycles and high plasticity, but also thanks to human factors such as habitat degradation and the strong propagule pressure associated with its ornamental interest.

Índex

Abreviatures

ABA	Àcid abscísic
Chl *a/b*	Relació clorofil·la *a* i *b*
DPS	Estat de de-epoxidació de les VAZ
ETR	Taxa de transport electrònic
F_v/F_m	Eficiència quàntica màxima del fotosistema II
JA	Àcid jasmònic
ROS	Espècies reactives de l'oxigen
SA	Àcid salicílic
SDM	Model de distribució de l’espècie
VAZ	Xantofil·les violaxantina, anteraxantina i zeaxantina
Φ_{PSII}	Eficiència quàntica relativa del fotosistema II

Introducció general

Introducció general

1. La biodiversitat: beneficis i amenaces

1.1. Importància de la biodiversitat

El Conveni de les Nacions Unides sobre la Diversitat Biològica (CBD de l'anglès *Convention on Biological Diversity*, celebrat a Rio de Janeiro el 1992) defineix la biodiversitat o diversitat biològica com la variabilitat d'organismes vius i els complexos ecològics dels quals formen part, incloent la diversitat dins de cada espècie, entre les espècies i els ecosistemes. Ramon Margalef va ser un important contribuïdor a la definició i quantificació en termes d'unitats d'informació de la biodiversitat, definint-la com el diccionari que utilitza la vida per construir-se en unitats funcionals tals com les cèl·lules, individus, poblacions, comunitats i ecosistemes (Margalef, 1957).

La biodiversitat és essencial per al manteniment de les funcions i serveis ecosistèmics dels quals ens beneficiem i són indispensables per al manteniment de l'homeòstasi terrestre (Cardinale et al., 2012). Pel que fa a la biodiversitat vegetal, en depenem directament per l'alimentació i els materials de construcció, o indirectament a través dels serveis ecosistèmics que proveeixen com per exemple la producció d'oxigen, la fixació de carboni, la reducció de la temperatura i fins i tot la millora del benestar. Tant per l'agricultura, la ramaderia, el sector turístic o com font de fàrmacs, la biodiversitat té un gran valor d'ús. Ara bé, el valor de la biodiversitat va més enllà de l'ús directe que puguem fer de les espècies, productes o molècules clau que podem obtenir-ne. Aquest valor deslligat de la utilització és el que anomenem valor intrínsec de la biodiversitat i inclou els serveis ecosistèmics que proveeix, entre els quals trobem l'estabilitat enfront de les pertorbacions a través de la relació diversitat - estabilitat (Elton, 1958). Tant la resistència o capacitat d'evitar la pertorbació com l'elasticitat o la velocitat de recuperació després de la pertorbació són superiors en comunitats amb major biodiversitat (Tilman et al., 2006). La biodiversitat ens protegeix també a nosaltres de les pertorbacions, així com de l'aparició i de la transmissió de malalties infeccioses (Keesing et al., 2010).

La preservació de la biodiversitat és necessària per a l'assoliment dels objectius de desenvolupament sostenible (ODS) definits per l'Assemblea General de Nacions Unides a complir pel 2030. La pèrdua de biodiversitat amenaça l'acompliment dels ODS 14 i 15 (relacionats amb la preservació de la vida submarina i terrestre, respectivament) però també altres objectius sobre els quals la pèrdua de biodiversitat podria impactar de forma indirecta, com en la fam zero (ODS 2) i en la salut i el benestar (ODS 3).

1.2. La biodiversitat al Mediterrani

Les 1,4 milions d'espècies que s'han identificat a la Terra no es distribueixen de forma igual en la superfície. Per exemple, els tròpics acumulen major biodiversitat (Fischer, 1960; Lawrence i Fraser, 2020). A banda de l'increment de biodiversitat a baixes latituds, hi ha zones del planeta que mostren grans concentracions en el nombre d'espècies. Aquestes regions, que anomenem punts calents de biodiversitat, es caracteritzen per contenir com a mínim 1.500 espècies vasculars natives i haver perdut almenys un 70 % de la vegetació original nativa (Myers et al., 2000). Es reconeixen 34 punts calents de biodiversitat que en conjunt agrupen un 50 % de les espècies vegetals i un 42 % dels vertebrats terrestres a escala mundial (Mittermeier et al., 2004) (**Figura 1**).

Entre les grans unitats de classificació dels paisatges de la biosfera (biomes) trobem que el bioma mediterrani constitueix la llar d'un nombre globalment significatiu i remarcable d'espècies vegetals i endemismes (Esler et al., 2018). Els ecosistemes de tipus mediterrani contenen un 20 % de totes les espècies de plantes vasculars en només un 2 % de l'àrea mundial terrestre i per aquest motiu són considerats punts calents de biodiversitat des de finals dels anys noranta i són una prioritat de conservació global (Myers et al., 2000; Mittermeier et al., 2004; Matesanz i Valladares, 2014). Entre les regions de clima mediterrani, la conca Mediterrània delimita el punt calent de diversitat més extensiu, amb 25.000 espècies natives (Myers et al., 2000), i constitueix el segon punt calent de biodiversitat més gran, amb un 20 % d'espècies en perill d'extinció (Myers et al., 2000).

L’origen d’aquesta gran biodiversitat és divers. Per una banda, la localització geogràfica juga un paper important. La conca Mediterrània es troba a la intersecció entre tres grans masses de terra: Europa, Àsia i Àfrica i inclou més de 34 països. Per altra banda, el seu nínxol climàtic estacional (**Figura 1**) dona lloc a la inusual dissociació temporal durant l’any entre les temperatures òptimes per al creixement i la baixa disponibilitat hídrica durant l’estiu, fet que provoca l’ampli desplegament dels mecanismes de fotoprotecció (Fernández-Marín et al., 2017). Els estius i els hiverns mediterranis són els períodes més estressants per a la vegetació en aquests ambients, ja que, d’una banda, a l’estiu hi ha períodes de sequera variables en durada i intensitat, i de l’altra, els hiverns són freds però suaus (Mitrakos, 1982; Köppen, 1923).

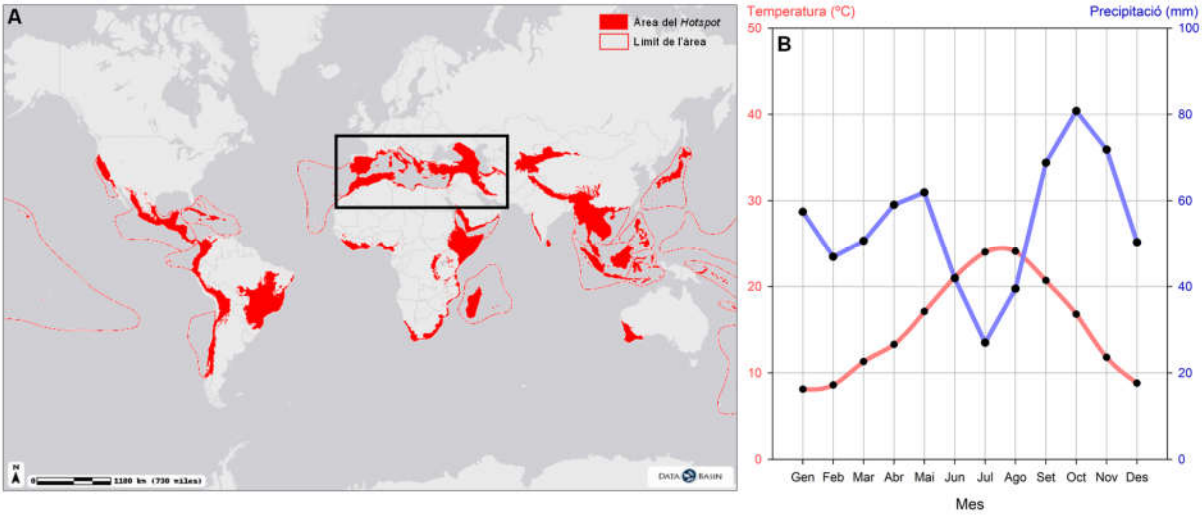

Figura 1. A. Punts calents de Biodiversitat, entre els quals trobem la conca Mediterrània (requadre negre) (Biodiversity hotspots 2011, www.databasin.org). **B.** Climograma mediterrani corresponent a la població de Roses (Dades climàtiques extretes de https://climatecharts.net/).

1.3. Amenaces a la biodiversitat

El creixement poblacional i la industrialització lligada al creixement econòmic han portat l'home a ocupar pràcticament tots els biomes, fet que ha impactat greument en la diversitat dels sistemes naturals (Otero et al., 2020). La globalització i l'elevada presència i densitat d'activitats humanes sobre la Terra han provocat taxes anormals d'extinció d'espècies (Otto, 2018). S'estima que al voltant de 2,3 espècies de plantes s'han extingit anualment en els darrers 250 anys, però és encara més alarmant el fet que la taxa s'ha doblat en els darrers 100 anys (Humphreys et al., 2019).

L'informe del 2019 resultant de l'avaluació global sobre la biodiversitat i els serveis dels Ecosistemes desenvolupat pel Panell Intergovernamental sobre Diversitat Biològica i Serveis dels Ecosistemes (IPBES, de l'anglés *Intergovernmental Platform for Biodiversity and Ecosystem Services*) va alertar que al voltant d'un milió d'espècies d'animals i plantes es troben actualment en risc d'extinció i que la salut dels ecosistemes es deteriora a una velocitat mai vista. A més, l'informe identifica com a principals agents impulsors de la degradació els canvis en l'ús de la terra i del mar, l'explotació directa d'organismes, el canvi climàtic, la contaminació i les espècies invasores. L'informe de l'IPBES projecta que una cinquena part de la superfície terrestre, incloent punts calents de biodiversitat, estan en risc degut a invasions biològiques (IPBES, 2019). Alguns dels agents impulsors de la pèrdua de biodiversitat tals com les espècies invasores, no només provoquen la pèrdua d'espècies, sinó que també causen la destrucció i degradació de la funcionalitat dels ecosistemes, la baixa resistència i resiliència a esdeveniments pertorbadors (Simberloff et al., 2013). A més, les espècies invasores poden actuar sinèrgicament amb altres elements del canvi global, incloent canvi d'usos del sòl (Hobbs 2000), canvi climàtic (Giejsztowt et al., 2020), increments en la concentració de CO_2 atmosfèric i la deposició de nitrogen (Weltzin et al., 2003).

Les espècies invasores constitueixen una amenaça global, però algunes regions del món podrien ser més susceptibles als seus impactes considerant la sinergia amb altres agents de canvi. A les zones amb ecosistemes de tipus mediterrani, degut a la gran acumulació d'espècies, es preveu una gran sensibilitat a canvis en els règims climàtics en el context del canvi global, i que això impacti greument en la biodiversitat d'aquestes regions (Esler et al., 2018). El canvi climàtic tindrà un greu impacte sobre les regions mediterrànies (Klausmeyer i Shaw, 2009). Pel 2050 s'esperen temperatures un 35-40 % superiors amb relació als nivells globals actuals, en paral·lel a les prediccions de reduccions en la precipitació (Giorgi, 2006; Christensen et al., 2007; Sheffield i Wood, 2008). Per tot això, la conca Mediterrània ha estat definida com un dels punts calents de sensibilitat al canvi climàtic i representa, per tant, una àrea prioritària per a la conservació de la biodiversitat global, on les espècies invasores són una de les amenaces principals (Giorgi, 2006).

2. Les espècies invasores

2.1. Definicions

La Unió Internacional per la Conservació de la Natura (IUCN, de l'anglès *International Union for Conservation of Nature*) estableix que les espècies invasores o espècies exòtiques invasores són aquelles introduïdes per l'home a emplaçaments fora el seu rang o distribució natural, on s'estableixen i es dispersen extensament, generant un impacte negatiu en l'ecosistema local i en les espècies autòctones. Charles S. Elton (1900-1991) és considerat el pare de l'estudi sistemàtic de les invasions biològiques. Arran de la publicació del seu llibre "L'ecologia de les invasions animals i vegetals" l'any 1958, el nombre de publicacions sobre l'ecologia de les invasions ha crescut ininterrompudament, amb una tendència exponencial a partir del 1980, moment en què es va formar el comitè SCOPE (de l'anglès *Scientific Comittee on Problems of the Environment*), el qual alertava de l'amenaça d'aquestes espècies (Richardson i Pyšek, 2008). Com a disciplina relativament jove, el marc de treball de les invasions biològiques és encara dinàmic, especialment per la creixent tendència a la interdisciplinarietat (Vaz et al., 2017). Alguns investigadors presenten la invasió com un procés per estadis

(Richardson et al., 2000; Colautti i MacIsaac, 2004; Catford et al., 2009; Blackburn et al., 2011). Aquests estadis estan delimitats per barreres o filtres ecològics que les espècies han de travessar per arribar al següent estadi (Richardson et al., 2000; Mitchell et al., 2006; Theoharides i Dukes, 2007). Blackburn et al. (2011) va plantejar un marc de treball unificat per a l'estudi de les invasions, recapitulant els conceptes de barreres i filtres ecològics en els estadis espaciotemporals de la invasió, terminologia i estratègies de gestió, tant en plantes com en animals, que derivaven dels models proposats de Richardson et al. (2000) i Williamson i Fitter (1996), respectivament. Quan una espècie s'introdueix fora del seu rang de distribució natural, travessant la barrera geogràfica a través del transport, es considera una espècie no nativa o exòtica. Si una espècie exòtica troba els factors biòtics i abiòtics per poder establir-se i créixer al medi on ha estat introduïda es considera una espècie exòtica aclimatada, ja que ha superat la barrera ambiental a través de la introducció al medi adequat. Activitats com el cultiu o creixement en captivitat poden promoure aquesta introducció (Bock et al., 2018). Si l'espècie és capaç de propagar-se superant la barrera reproductora a través de la colonització, es parla d'una espècie naturalitzada o establerta. En el cas que l'espècie s'expandeixi de forma ràpida i generi un impacte negatiu sobre els ecosistemes, serà llavors quan parlarem d'espècie invasora, superant la barrera dispersiva.

De forma paral·lela a la complexa definició ecològica d'espècie invasora, en l'àmbit legal ha calgut definir unitats concretes sobre les quals planificar mesures de prevenció, control i erradicació. L'aproximació escollida ha estat la definició de llistes o catàlegs d'espècies exòtiques invasores d'acord amb seu impacte en un territori concret. A escala mundial, el Conveni de Nacions Unides sobre la Diversitat Biològica va reconèixer l'existència de la problemàtica de les espècies invasores i va establir que cada part contractant, en la mesura del possible, impedirà que s'introdueixin i controlarà o erradicarà les espècies exòtiques que amenacen els ecosistemes, els hàbitats o les espècies. A Espanya, la definició legal d'espècie invasora ve delimitada per la catalogació dins del Catàleg espanyol d'espècies exòtiques invasores, regulat pel Reial Decret 630/2013, de 2 d'agost que tenia origen en l'article 64 de la llei 42/2007 de 13 de desembre, del Patrimoni Natural i de la Biodiversitat. Actualment (incloent les modificacions realitzades el 2019 a través del Reial Decret 216/2019 de 29 de març), el catàleg inclou fongs (1 taxó), algues (14 taxons), plantes (67 taxons), invertebrats (43 taxons), peixos (21 taxons), amfibis (4 taxons), rèptils (7 taxons), aus (17 taxons) i mamífers (16 taxons).

2.2. Impactes globals

Els impactes derivats d'espècies invasores són de difícil mesura degut a que afecten diferents nivells (Crystal-Ornelas i Lockwood, 2020). Impacten sobre el funcionament dels ecosistemes, l'economia i, en alguns casos, també directament sobre la nostra salut (Kumar Rai i Singh, 2020).

Pel que fa al funcionament dels ecosistemes, actuen a través de tres mecanismes: (a) reducció en la biodiversitat nativa, (b) alteracions en les propietats fisicoquímiques del sòl, i (c) estimulació de la resposta de l'ecosistema cap a règims de pertorbacions alterats (Pyšek et al., 2012). Aquests tres mecanismes actuen sovint de forma coordinada. Per exemple, la invasió d'*Acacia dealbata* al Mediterrani provoca l'alteració de cicles de nutrients, propietats fisicoquímiques del sòl i canvis en l'activitat microbiana, fet que provoca la reducció de la biodiversitat nativa (Lazzaro et al., 2014). La invasora *Robinia pseudoacacia* exerceix una atracció als pol·linitzadors més forta que la nativa *Cytisus scoparius*, obstaculitzant la pol·linització d'aquesta (Buchholz i Kowarik, 2019).

Només als Estats Units, l'impacte estimat de les espècies invasores és de 31 mil milions anuals (Pimentel et al., 2005). Aquestes espècies causen una pèrdua en el rendiment econòmic potencial d'activitats humanes tals com l'agricultura, la pesca i fins i tot el turisme, així com danys directes sobre les infraestructures. Per exemple, *Fallopia japonica* a Anglaterra danya carreteres, vies de tren i edificis, el qual suposa un cost de gestió i erradicació de 184 milions d'euros anuals (Williams et al., 2010). Les despeses del combat contra les invasores no només inclouen l'erradicació, sinó també les mesures necessàries d'aïllament, detecció precoç i control. Malgrat que la introducció d'algunes espècies exòtiques en alguns casos respon a interessos econòmics, sovint el resultat ha estat un balanç econòmic global negatiu i una devaluació dels ecosistemes (Kumar Rai i Singh, 2020).

A banda dels impactes sobre la biodiversitat i l'economia, algunes espècies invasores impacten sobre la nostra salut de forma directa a través de l'alliberament de toxines o compostos al·lergògens, o actuant com a vectors de malalties. També impacten de forma indirecta a través de la pèrdua de biodiversitat, degradant així el seu rol protector. Algunes espècies invasores constitueixen vectors de malalties, com és el cas de *Parthenium hysterophorus*, vector de la malària (Nyasembe et al., 2015; Stone et al., 2018). Altres invasores alliberen toxines químiques, com *Rhododendron ponticum*, que contamina la mel amb toxines que causen problemes de salut humana (Koca i Koca, 2007). Tal com recull Kumar Rai i Singh (2020), un gran nombre d'espècies invasores a Europa, entre les quals trobem *Ambrosia artemisiifolia*, *Ailanthus altissima* o espècies del gènere *Eucalyptus*, causen al·lèrgia en humans. La invasora *Opuntia stricta* conté compostos en el fruit que provoquen irritació ocular (Shackleton et al., 2017). Espècies invasores com *Cortaderia selloana* (Mazza et al., 2014), tal com suggereix el mateix nom, ocasionen dany físic tal com talls deguts a la seva morfologia foliar.

2.3. Què fa que una espècie exòtica esdevingui invasora?

El motiu pel qual algunes espècies esdevenen invasores ha estat discutit a partir de diverses hipòtesis i actualment encara no s'ha arribat a definir un mecanisme únic i concret. Diversos autors han intentat integrar les diferents hipòtesis existents i cada cop n'han posat més sobre la taula. Per exemple, Catford et al. (2009) proposava integrar 29 hipòtesis, Enders et al. (2018) proposava integrar-ne 33, Enders et al. (2019) en proposava 35 i molt recentment Enders et al. (2020) en proposa 39. Amb relació a aquest nombre aclaparador i creixent d'hipòtesis, en l'estudi de la invasió biològica s'identifiquen quatre grans dificultats: (1) no hi ha claredat en els conceptes centrals i hipòtesis, (2) algunes hipòtesis tenen diferents noms però representen el mateix, (3) algunes hipòtesis es contradiuen i hi ha confusió sobre si tenen suport empíric i (4) hi ha hipòtesis sense suport empíric que se segueixen utilitzant (Jeschke, 2014; Enders et al., 2018). L'estudi d'Enders et al. (2018) va preguntar a més de 350 investigadors en el camp de la invasió biològica sobre les diferents hipòtesis, mostrant que no hi ha un mapa clar a les ments dels investigadors que connecti les diferents hipòtesis. Malgrat això, sí que es van identificar les hipòtesis més destacades i comunitats d'hipòtesis al voltant d'aquestes. Conjuntament amb l'estudi d'Enders et al. (2019), que presenta una anàlisi de co-citacions que permet veure les relacions entre les diferents hipòtesis, podem identificar grans famílies d'hipòtesis per explicar les invasions biològiques. Aquests clústers amb petites variacions també apareixen en analitzar les diferents hipòtesis amb un mètode Delphi que permet copsar l'expertesa dels investigadors i evitar el biaix de les cites (Enders et al., 2020). Així doncs, actualment s'identifiquen els següents grups d'hipòtesis que explicarien l'èxit invasor: hipòtesis d'interaccions biòtiques, hipòtesis de Darwin, hipòtesis dels propàguls, hipòtesis de la disponibilitat de recursos i hipòtesis dels atributs. Dins de cada clúster, les hipòtesis més acceptades i amb més suport empíric seguint les propostes de Catford et al. (2009), Jeschke (2014), Rejmánek (2014), Jeschke i Heger (2018) i Enders et al. (2020) són:

Clúster d'hipòtesis d'interaccions biòtiques: reuneix les hipòtesis que assumeixen que els enemics naturals controlen les poblacions. La hipòtesi amb major suport és l'alliberament dels enemics naturals, que planteja que l'èxit invasor s'explica per l'absència d'enemics naturals en el lloc d'arribada alliberant les espècies de condicions estressants (Blossey i Notzold, 1995). Per exemple, s'associa l'èxit d'*Impatiens glandulifera* amb l'alliberament de patògens fúngics a les llavors de l'espècie en el rang introduït (Najberek et al., 2018). Alguns estudis presenten el contrapunt d'aquesta hipòtesi demostrant que algunes espècies exòtiques tenen més enemics naturals nous al rang invasor (Verhoeven et al., 2009). Una segona hipòtesi amb fort suport és la hipòtesi de l'evolució d'una major habilitat competitiva que pot ser deguda a processos de deriva genètica per efecte fundador dels pocs individus que arriben al rang invasor (Bossdorf et al., 2005). La comparació entre poblacions natives i invasores en *Spartina alternifolia* mostra com els individus del rang invasor presenten un avançament en la fenologia, un major creixement i inversió en biomassa dels rizomes, suggerint que l'augment en la reproducció asexual contribueix a l'èxit de la invasió (Shang et al., 2015).

Clúster d'hipòtesis de Darwin: agrupa les hipòtesis que aborden les relacions d'ecologia evolutiva i filogènia entre espècies exòtiques i natives. Alguna de les hipòtesis amb major adhesió és la de la resistència biòtica també coneguda com la hipòtesi de diversitat-invasibilitat que postula que aquells ecosistemes amb elevada biodiversitat seran més resistents a la invasió (Elton, 1958; Levine i D'Antonio, 1999). També s'han presentat hipòtesis referents a la facilitat d'invasió quan la invasora és molt diferent de les espècies natives (hipòtesi de la semblança limitant), o bé hi ha nínxols buits (Levins i Macarthur, 1967; MacArthur, 1970).

Clúster d'hipòtesis dels propàguls: agrupa les hipòtesis que relacionen el nombre d'espècies exòtiques amb la probabilitat que esdevinguin invasores, des d'un punt de vista poblacional. La regla del deu o *Ten's rule* és una de les hipòtesis incloses en aquest clúster i postula que la probabilitat que una espècie creui cadascuna de les barreres o filtres de la invasió és del 10 % en cada cas, basant-se en l'assumpció que algunes comunitats són més fàcils d'envair que altres (Williamson i Brown, 1986; Richardson i Pyšek, 2006). Un major nombre de propàguls permetria superar l'estocasticitat i garantir l'establiment i persistència de les poblacions (Lockwood et al., 2005). Una altra hipòtesi amb relació als propàguls és la de l'esfondrament invasor, la qual postula que la invasió és major en ecosistemes amb presència prèvia d'altres espècies invasores (Simberloff i von Holle, 1999; Richardson i Pyšek, 2006).

Clúster d'hipòtesis de la disponibilitat de recursos: reuneix les hipòtesis que associen l'èxit invasor amb l'accés als recursos. Una de les hipòtesis amb més suport és la de l'elevada disponibilitat de recursos, que postula que l'èxit de les espècies invasores augmenta amb la disponibilitat de recursos (Sher i Hyatt, 1999). Amb relació a aquesta hipòtesi també es destaca la hipòtesi del règim de pertorbacions, que postula que la invasió és major en ecosistemes degradats (Elton, 1958; Hobbs i Huenneke, 1992). Ara bé, la revisió de Funk (2013) exposa com les invasores també poden ser exitoses en ambients amb baixa disponibilitat de recursos.

Clúster d'hipòtesis dels atributs: agrupa les hipòtesis que estableixen que els atributs d'una espècie determinen que esdevingui o no invasora. Per exemple, la hipòtesi de l'invasor ideal postula que l'èxit d'una espècie invasora depèn dels seus atributs específics (Baker, 1965). Malgrat que no s'han identificat característiques comunes en totes les espècies que defineixin aquest "invasor ideal", grans inversions en reproducció, major altura, cicles de vida curts, la formació d'un banc de llavors o el creixement clonal són alguns dels atributs proposats per a definir-lo (Bossdorf et al., 2005; Richardson i Pyšek, 2006; Pyšek i Richardson, 2007; Pyšek et al., 2009, 2015; van Kleunen et al., 2010; Moravcová et al., 2015; Gioria i Pyšek, 2016). El creixement clonal ha estat considerat com un atribut comú en moltes plantes invasores (Pysek, 1997; Song et al., 2013), ja que algunes característiques clau com la integració fisiològica o la divisió de tasques permetrien a aquestes espècies envair un ampli espectre d'hàbitats (Pysek, 1997; Roiloa, 2019). També els atributs ornamentals s'han proposat com a beneficiosos per la invasió a través de l'home, propiciant múltiples introduccions i una elevada pressió de propàguls (Reichard i White, 2001). Aquest clúster també inclou la hipòtesi de similitud climàtica o filtratge d'hàbitat, que planteja que les invasores seran exitoses si estan preadaptades a les condicions del rang introduït (Weiher i Keddy, 1995). L'assumpció de la conservació del nínxol des del rang natiu al rang introduït s'ha utilitzat per a delimitar les àrees amb probabilitat a ser envaïdes i valorar el risc invasor a través de models de distribució d'espècies (SDM, de l'anglès *species distribution model).* Finalment, una altra hipòtesi d'aquest clúster és que l'èxit de les espècies invasores deriva d'una major plasticitat fenotípica (Richards, 2006).

3. Ecofisiologia de les invasions vegetals

L'ecofisiologia vegetal es defineix com una ciència experimental que persegueix els mecanismes fisiològics per donar resposta a qüestions ecològiques relacionades amb la supervivència, el creixement, la distribució geogràfica, l'abundància i les interaccions de les plantes amb altres organismes, considerant la relació que guarden amb el seu ambient físic, químic i biòtic (adaptat de Lambers i Oliveira, 2019). Des d'una perspectiva ecofisiològica, la presència d'una espècie en un indret específic respon a la capacitat dels individus de resistir, evitar o tolerar les alteracions en l'ambient físic, químic i biòtic que els envolta i reproduir-se exitosament tot assegurant la persistència. L'expansió d'una espècie en noves àrees vindrà determinada pels atributs del flux de propàguls, però també per la capacitat de l'espècie d'aclimatar-se, i a la llarga, d'adaptar-se a unes noves condicions ambientals.

Algunes de les hipòtesis d'invasió proposen associar l'èxit d'una espècie entorn de la seva capacitat de fer front a les condicions ambientals. Una major capacitat de créixer sota determinades condicions ambientals, fent front als estressos associats podria ser determinant per a l'èxit de la invasió (Pintó-Marijuan i Munné-Bosch, 2013). La hipòtesi més acceptada, l'alliberament d'enemics naturals s'ha demostrat amb diverses espècies que provoca una redistribució dels recursos defensius i promou que es destini més partició energètica al creixement i reproducció. Aquest major vigor en espècies invasores al seu rang d'introducció, en suposar un avantatge competitiu, seria afavorit per la pressió de selecció. Tant la bioquímica de la planta (Inderjit et al., 2006) com la seva fisiologia, en termes de l'eficiència de l'ús dels recursos com ara nutrients, aigua o llum, són determinants per a la superioritat competitiva de les espècies invasores, especialment en hàbitats amb recursos limitats (Funk, 2013). La capacitat de tolerar una gran varietat de condicions ambientals és determinant en l'èxit invasor (Higgins i Richardson, 2014).

Des d'aquesta perspectiva ecofisiològica, la supervivència d'una espècie en un indret ve determinada per les relacions entre la tolerància a condicions adverses, la reproducció i la mort, és a dir, la seva estratègia vital.

3.1. Estrès

L'estrès és un pilar bàsic en l'ecofisiologia vegetal. En el context de l'ecofisiologia definim l'estrès com el conjunt de factors ambientals que redueixen la taxa d'algun procés fisiològic per sota del màxim que una planta podria d'altra manera tenir (Lambers i Oliveira, 2019). A partir de definicions del camp de la física, Levitt (1980) va definir l'estrès com la deformació d'un cos a l'aplicar una pressió, que en intensificar-se pot ser reversible (elàstica) o irreversible (plàstica) fins a arribar al trencament. La duració de l'estrès, la intensitat, la capacitat de reparació i d'adaptació, la preexposició o la presència de múltiples estressos condicionen el resultat final de l'estrès. Les característiques de la planta també contribuiran a aquest resultat final: la part de la planta afectada, la fase de creixement o el genotip determinaran si l'individu resisteix i sobreviu, o bé si és sensible al factor ambiental.

Els mecanismes de resposta a l'estrès es basen en tres grans estratègies: escapar, evitar i tolerar (Ludlow, 1989; Lambers i Oliveira, 2019). L'estratègia d'escapar implica evitar estar present durant les condicions desfavorables i és una estratègia típica d'espècies amb cicle vital curt, en què són les llavors les que passen l'època desfavorable. Si en comptes d'escapar es resisteix, distingim les estratègies d'evitar i tolerar. L'estratègia d'evitar implica no permetre que les condicions estressants afectin la fisiologia de la planta, mentre que tolerar implica resistir les condicions sense executar modificacions per evitar-les. Aquestes respostes de resistència poden ser adquirides per adaptació o per aclimatació. L'adaptació és un mecanisme constitutiu, adquirit durant l'evolució de la planta i és heretable. L'aclimatació contempla aquells mecanismes ràpids en el metabolisme induïts per l'estrès que en principi no seran heretats a les següents generacions, però que contribueixen a la supervivència de l'individu.

Com a organismes sèssils, les plantes han de sobreviure a les condicions ambientals locals, però també han d'estar preparades per respondre a períodes d'estrès derivats dels canvis i dinàmiques naturals d'aquestes condicions. L'estrès fotooxidatiu és central en l'optimització entre l'adquisició i utilització de l'energia en plantes i dona lloc a la capacitat d'adaptació i aclimatació a l'ambient (Demmig-Adams et al., 2014). El tancament estomàtic és una resposta comuna a diferents estressos, donant lloc a taxes d’assimilació de CO_2 subòptimes, de manera que l’absorció de llum pot superar la capacitat fotosintètica (Takahashi i Badger, 2011). En aquestes condicions i també en condicions d’elevada radiació o la falta d’algun element fonamental per la planta, hi ha un excés d’energia al cloroplast (Demmig-Adams et al., 2014; Demmig-Adams et al., 2017). La saturació de la cadena de transport electrònic que permet l’acumulació d’energia necessària per a la fotosíntesi porta a una acumulació de molècules de clorofil·la excitades ($^3Chl^*$), que alhora promou la formació d’espècies reactives de l’oxigen (ROS, de l’anglès *reactive oxygen species*) tals com el singlet d’oxigen ($^1O_2$), l’ió superòxid ($\cdot O_2^-$), el peròxid d’hidrogen (H_2O_2) o el radical hidroxil ($\cdot OH$) (Asada, 2006). Si les defenses antioxidants no aconsegueixen compensar aquesta generació de ROS, apareix l’estrès fotooxidatiu, que a la llarga podria provocar dany oxidatiu (Apel i Hirt, 2004). Un excés de ROS induiria, per exemple, la peroxidació dels lípids, provocant l’acumulació d’hidroperòxids lipídics (LOOH), un dels primers productes del dany que al mateix temps danyarien els fotosistemes (Pospíšil i Yamamoto 2017). Les ROS sobre els complexos proteics del tilacoide inhibeixen la reparació de la proteïna D1, que forma part del fotosistema II i és imprescindible per al correcte funcionament de la cadena de transport electrònic, que en degradar-se per l’excés de llum ha de ser sintetitzada de nou (Takahashi i Badger, 2011; Goh et al., 2012). Quan la taxa d’oxidació de la proteïna D1 supera la capacitat de regeneració, és quan parlem de fotoinhibició, una reducció de la capacitat fotosintètica induïda per la pròpia llum (Takahashi i Badger, 2011). En condicions de foscor, si no hi ha fotoinhibició crònica, tots els centres de reacció es troben reparats i s’estima que l’eficiència quàntica màxima del fotosistema II (F_v/F_m) es troba al voltant de 0,8 (Demmig i Björkman, 1987). Una menor F_v/F_m reflecteix la destrucció d’alguns fotosistemes i, per tant, una ineficiència en el processament de la llum degut a una major degradació de la proteïna D1. En condicions de llum, l’eficiència cau degut a la saturació dels centres de reacció (Demmig-Adams i Adams, 2018). Definim aquesta eficiència en condicions de llum com l’eficiència quàntica relativa del fotosistema II (Φ_{PSII}). Per corregir l’efecte de la quantitat de llum rebuda, sovint s’expressa aquesta eficiència en termes de la taxa de transport electrònic a través de la cadena de transport d’electrons

(ETR, de l'anglès *electron transport rate*). Aquesta taxa es basa en la quantitat de llum fotosintèticament activa rebuda o PAR (de l'anglès *photosynthetically active radiation*), un factor de 0,5 pel fet que només considerem el fotosistema II, i el coeficient d'absorbància de la fulla, que s'estima al voltant de 0,84. Així doncs, el càlcul de l'ETR es determina amb la següent fórmula: $ETR = \Phi_{PSII} * PAR * 0,5 * 0,84$.

Per tal d'evitar l'excés de llum i, en darrer lloc, el dany oxidatiu sobre les molècules, la planta disposa de diferents nivells de fotoprotecció. En primer lloc, la radiació es pot evitar amb el moviment de les fulles o dels cloroplasts, així com a través de la producció de compostos fenòlics que fan de pantalla de la radiació UV (Takahashi & Badger, 2011). Com a col·lectores de la llum, les clorofil·les constitueixen un element regulador central de l'acumulació d'energia al cloroplast i responen a una gran varietat d'estressos (Esteban et al., 2015). Un cop la llum ha arribat als centres de reacció, altres mecanismes fotoprotectors guanyen importància. Els carotenoides són un grup d'isoprenoides ubics dins del regne vegetal (Esteban et al., 2009; Nisar et al., 2015) que tenen un doble rol en la maquinària fotosintètica, ja que protegeixen del dany fotooxidatiu, però també són pigments accessoris als centres de captació de llum (Yamamoto i Bassi, 1996). Dins del grup dels carotenoides, s'hi distingeixen el grup dels carotens (on el β-carotè és el més abundant) i el de les xantofil·les, les quals contenen oxigen en la seva estructura (com la luteïna, violaxantina, zeaxantina, anteraxantina i neoxantina). El β-carotè és especialment eficient capturant el singlet d'oxigen generat en el fotosistema II, a conseqüència de l'excés de llum (Ramel et al., 2012). La luteïna és la xantofil·la més abundant en plantes i és essencial dissipant l'energia del triplet de clorofil·la excitat ($^3Chl^*$), però és també un component estructural clau dels complexos proteics dels fotosistemes I i II (Dall'Osto et al., 2006). A més, altres xantofil·les són clau com a dissipadores físiques a través del cicle de les xantofil·les o cicle VAZ (Demmig-Adams i Adams, 1992; 1996). La de-epoxidació de violaxantina (Vx) a anteraxantina (Ax) i zeaxantina (Zx), és a dir, la dissipació tèrmica o dissipació no fotoquímica (NPQ) (també mesurada com a estat de de-epoxidació o DPS), respon a diferents estressos ambientals (Demmig-Adams i Adams, 1996).

La neutralització de les ROS a través de molècules d'antioxidants constituiria la següent línia de defensa per evitar l'estrès i dany oxidatiu, reaccionant químicament amb les ROS per eliminar-les o bé dissipant la seva energia a través d'un procés físic. La tolerància de les plantes a l'estrès fotooxidatiu requereix mecanismes que protegeixin els lípids, ja que, que degut a la seva elevada insaturació, són molt sensibles a l'oxidació i són sovint un dels primers signes d'estrès fotooxidatiu (Douce i Joyard, 1980). Per la seva estreta relació amb l'estrès fotooxidatiu, i el seu rol en la protecció dels lípids, destaquen els tococromanols o vitamina E com a antioxidants lipòfils no enzimàtics importants en la resposta a l'excés d'energia al cloroplast, cedint electrons a ROS com ·OH i $^1O_2$ (Munné-Bosch i Alegre, 2002; Triantaphylidès i Havaux, 2009). Els tococromanols tenen un paper fonamental en la prevenció de la propagació de la peroxidació lipídica, trobant-se en majors concentracions en plantes adaptades a la sequera o a altres estressos (Munné-Bosch i Alegre, 2002). L'α-tocoferol és l'homòleg majoritari en fulles, seguit del seu precursor, el γ-tocoferol (Falk i Munné-Bosch, 2010).

La fotoprotecció i l'estrès oxidatiu reflecteixen l'estat fisiològic de la planta i també el potencial d'aquesta per donar resposta a la variabilitat en les condicions ambientals. Les hormones vegetals o fitohormones tenen un paper clau en el desenvolupament vegetal, però són també les responsables de desencadenar aquestes respostes adaptatives en condicions d'estrès, actuant com a missatgers químics (Verma et al., 2016). De forma molt general, les hormones clàssicament associades amb les respostes a l'estrès són l'àcid abscísic (ABA), l'àcid salicílic (SA) i l'àcid jasmònic (JA). Les tres tenen rols ben coneguts en la fisiologia vegetal. L'àcid abscísic és el responsable del tancament estomàtic ja que regula el flux iònic a les cèl·lules oclusives (Kim et al., 2010). Les hormones SA i JA s'han relacionat tradicionalment amb la resposta a patògens i herbívors, malgrat que també tenen un paper en les respostes a l'estrès abiòtic. Per exemple, s'ha descrit que el SA, a banda d'induir la resistència sistèmica adquirida en resposta a patògens, contribuiria a mantenir l'activitat fotosintètica sota condicions d'estrès abiòtic (Scott et al., 2004; Soliman et al., 2018). En el cas del JA, a més de respondre al dany físic i a atacs de patògens, participa en nombrosos processos en interacció amb altres hormones com la tolerància a l'estrès abiòtic, la formació d'òrgans de reserva o l'elongació de l'arrel (Ahmad et al., 2016). Altres hormones no clàssicament associades a l'estrès també participen en la senyalització d'aquest. Per exemple, l'etilè (ET) inhibeix l'elongació foliar i indueix la formació d'aerènquima com a adaptació a l'estrès per inundació (Kazan, 2015). També les citocinines, a banda d'estimular la divisió cel·lular i participar en els processos de remobilització de nutrients, contribueixen a mantenir la integritat dels cloroplasts (Cela et al., 2018) frenant els processos de senescència i afavorint la recuperació (Rivero et al., 2007). El fet que la definició d'hormona vegetal disti de la definició d'hormona animal ha fet que aquesta sigui sovint reavaluada i sigui encara motiu de debat. A part de les fitohormones clàssiques, també altres compostos s'han proposat com a reguladors hormonals, com el cas de la melatonina (N-acetil-5-metoxitriptamina), la funció de la qual és un tema obert a discussió i s'ha proposat tant com a reguladora del creixement com a molècula antioxidant (Arnao i Hernández, 2014; Gao et al., 2016).

3.2. Reproducció

La reproducció és un procés clau del cicle vital dels individus, ja que és el màxim contribuïdor a l'èxit de l'espècie. La reproducció juga un paper clau en les invasions vegetals durant les etapes de colonització, dispersió i expansió. Alguns dels atributs que s'han proposat per tal que una espècie exòtica esdevingui invasora impliquen aspectes de la reproducció. Per exemple una estratègia tipus “r” que equival a un cicle de vida ràpid amb elevada reproducció, o reproducció clonal s'han relacionat amb l'èxit invasor (Lodge, 1993; Bossdorf et al., 2005; Richardson i Pyšek, 2006; Pyšek i Richardson, 2007; Pyšek et al., 2009, 2015; van Kleunen et al., 2010; Moravcová et al., 2015).

Adaptativament les plantes han desenvolupat diferents estratègies reproductores. Segons la freqüència de l'esdeveniment reproductor podem categoritzar les espècies entre iteròpares i semèlpares. Les espècies semèlpares o monocàrpiques presenten un únic cicle reproductiu abans de la seva mort (Cole, 1954). Aquesta estratègia equivaldria a una estratègia explosiva o de *Big Bang* que maximitza assegurar l'èxit de la reproducció invertint-hi tots els recursos a costa de la vida del propi individu. Per contra, les espècies iteròpares o policàrpiques tenen la capacitat de desenvolupar múltiples cicles reproductius al llarg de la seva vida gràcies al manteniment d'un conjunt de meristemes apicals indeterminats i un conjunt de meristemes que esdevenen meristemes florals (Thomas et al., 2000). Des d'un punt de vista fisiològic, la reproducció de les plantes és un procés que comporta un esforç molt gran per a tot l'individu, ja que implica canvis a nivell de tot l'organisme i per tant porta uns costos associats (Obeso, 2002). Aquests costos són especialment visibles en espècies monocàrpiques, en què la reproducció suposa la mort de l'individu.

Factors ambientals com la disponibilitat de nutrients, la qualitat i quantitat de llum tenen efectes sobre l'estratègia reproductora de les plantes (van Baalen i Prins, 1983; Johnson, 2007; Williams, 2009). Aquesta plasticitat en l'estratègia reproductora sol tenir base genètica (Wesselingh et al., 1997; Johnson, 2007) i, per tant, és susceptible de respondre a la selecció i evolucionar de forma ràpida (Agrawal et al., 2013; Hahn i Müller-Schärer, 2013). L'expansió de les espècies invasores implica una situació de no equilibri que podria tenir un gran impacte sobre l'estratègia reproductora, degut a la presència de noves forces de selecció o a la manca d'enemics naturals, amb importants conseqüències demogràfiques (Aragón, 2014).

3.3. Longevitat i senescència

La mort es pot considerar la fi de la contribució de l'individu a la població, ja que frena el valor reproductor. Tant l'estrès com la reproducció guarden una relació molt forta amb la longevitat ja que regulen la contribució poblacional de l'individu. Si la resposta en front a les condicions ambientals no permet escapar, tolerar o resistir, l'estrès condueix a la mort de l'individu suposant la fi de la contribució reproductora (Ludlow, 1989).

Segons la longevitat de les plantes podem classificar-les en anuals, bianuals i perennes. Amb relació a la seva estratègia reproductora i a la seva longevitat podem classificar les espècies vegetals en anuals semèlpares, bianuals o perennes semèlpares i bianuals o perennes iteròpares. Les plantes anuals són aquelles que tenen un cicle vital d'un any de durada. Per definició són per tant també espècies semèlpares o monocàrpiques, ja que es reprodueixen un cop a la vida i la reproducció va associada a la mort de l'individu. Si el cicle de vida dura dos anys parlem de plantes bianuals, mentre que si el cicle de vida és superior als dos anys parlem de plantes perennes. Tant les plantes perennes com les bianuals poden ser semèlpares o iteròpares en funció de si es reprodueixen una sola vegada o diverses respectivament durant el seu cicle vital.

La mortalitat pot ser una conseqüència de factors interns tals com el programa de desenvolupament (que determina si són anuals, bianuals o perennes) o bé ser conseqüència de factors externs, com condicions ambientals subòptimes o pertorbacions (Quirino et al., 2000). La senescència en plantes és la darrera etapa abans de la mort d'un individu, un procés de desenvolupament acuradament programat i controlat a través de regulació hormonal (Jibran et al., 2013). L'objectiu final és desmantellar de forma òptima l'òrgan o la planta per tal de cedir els nutrients a les llavors o a teixits actius. La senescència es caracteritza per canvis estructurals, per un augment de les ROS, pel dany oxidatiu, per una davallada en la fotosíntesi i per una caiguda en el contingut de clorofil·les (Juvany et al., 2013). Un dels símptomes més visibles de la senescència és la pèrdua de la verdor associada a la degradació de clorofil·les, ja que el cloroplast és el primer a desmantellar-se, juntament amb la degradació de complexos proteics (Wingler et al. 2004). De forma molt controlada, durant el procés de senescència predominen els processos catabòlics i els nutrients alliberats són mobilitzats en estructures de reserva o dirigits a zones de creixement actiu com gemmes, fulles, fruits i llavors en desenvolupament. Tant en la senescència foliar com en la senescència a nivell de l'individu, l'inici del procés pot respondre tant al programa de desenvolupament de la planta com a factors ambientals tals com la sequera, deficiències de nutrients, la infecció per patògens o l'excés d'ombra (Quirino et al., 2000).

4. Model d'estudi: *Carpobrotus edulis* en la invasió del Mediterrani

Carpobrotus edulis (L.) N. E. Br. (Aïzoàcies) és una de les espècies invasores presents al Mediterrani i que, per tant, suposa una gran amenaça per a la biodiversitat d'aquest punt calent d'espècies natives. Reuneix algunes de les condicions que la podrien fer la "invasora ideal", ja que és una espècie amb creixement clonal, modifica les condicions fisicoquímiques del sòl i va acompanyada d'una elevada pressió de propàgul, ja que té interès ornamental. A més, la seva suculència suggereix que podria ser resistent a condicions més àrides en el context del canvi climàtic, consolidant-se així com una amenaça creixent per la biodiversitat del Mediterrani. Per tot això constitueix un model d'estudi per entendre el seu èxit a través d'una perspectiva ecofisiològica, integrant les respostes a l'estrès, la reproducció i la senescència.

C. edulis és una planta reptant suculenta amb una tija subcilíndrica i molt ramificada (**Figura 2**). Presenta fulles oposades, semiamplexicaules, erectes i amb secció triangular, lleugerament adnates a la base, de color verd brillant o lleugerament glauc sovint tenyides de vermell a les vores. A la perifèria de l'interior de la fulla es disposa una capa de clorènquima (cèl·lules de parènquima amb cloroplasts) amb teixit aquós a l'interior, que suposa el 69 % del pes fresc de la fulla i a través del qual creua el principal feix vascular (Earnshaw et al., 1987). Les flors són de color groc, rosat o porpra, de 8-10 cm de diàmetre amb tres tèpals externs i dos interns. Els aparents pètals són estaminodis petaloides (estams estèrils) linear-lanceolats disposats en 3-4 verticils. Presenta nombrosos estams amb els filaments groguencs. Té un ovari ínfer amb 8-16 carpels. Els estigmes són radials, subulats i plomosos. La fecundació és al·lògama i entomòfila. El fruit és una drupa carnosa, subglobosa de color groguenc, indehiscent i comestible. Les llavors, de 1-1,5 mm, són de color marró fosc (Sanz Elorza et al., 2004). S'ha descrit que aquestes llavors tenen una elevada longevitat, ja que podrien resistir viables fins a 5 anys després de la seva erradicació (Ruffino et al., 2015), generant un banc de llavors persistent (Gioria et al., 2012). La dispersió es realitza per via endozoocora, a través de petits mamífers tals com conills, rates o gats, així com babuïns o cérvols (Campoy et al., 2018). El genet (individu o conjunt d'individus originats a partir d'un mateix zigot) es reprodueix també activament per estolons que arrelen fàcilment en els nusos, formant un dens i fibrós sistema radicular concentrat en els 50 cm de sòl (D'Antonio i Mahall, 1991), donant lloc als diferents ramets o unitats vegetatives independents o amb capacitat de ser-ho.

C. edulis és originària de la República de Sud-àfrica, de la regió del Cap, però actualment es troba àmpliament distribuïda per les regions de clima mediterrani d'arreu del món (D'Antonio & Mahall, 1991). L'entrada d'aquesta espècie al continent europeu data de finals dels 1700 (Preston i Sell, 1988) i podria haver estat múltiple degut a l'alt interès ornamental de les Aïzoàcies a Anglaterra, França, Espanya, Itàlia i Portugal (Campoy et al., 2018). A més, l'espècie també va ser introduïda per a la fixació del sòl i recentment utilitzada i cultivada per interessos farmacològics. Creix especialment en zones costaneres, tant en zones rocoses com dunars, però en el rang invasor també és habitual en zones degradades, terraplens i marges de jardins (Lechuga-Lago et al., 2017). Ocupa tant zones obertes com sotabosc de comunitats arbòries mediterrànies (Traveset et al., 2008).

Malgrat que *C. edulis* ens pot proveir serveis ecosistèmics beneficiosos com són l'ús com a cultiu ornamental, l'estabilització del sòl, l'ús en medicina tradicional o per l'alimentació, genera un impacte negatiu sobre la biodiversitat nativa i contribueix a l'alteració de l'hàbitat (Milanovic et al., 2020). La invasió de *C. edulis* va associada a una disrupció dels processos biogeoquímics del sòl, alterant el pH, la salinitat, la humitat, el contingut de nutrients del sòl i la composició del microbiota del sòl (Conser i Connor, 2009; Novoa et al., 2014; Vieites-Blanco i González-Prieto, 2018a; Vieites-Blanco i González-Prieto, 2018b, Rodríguez-Caballero et al., 2020). La necromassa de *C. edulis*, que roman al sòl durant molts anys, allibera compostos al·lelopàtics que inhibeixen la germinació d'espècies natives dunars (Novoa i Gonzalez, 2014). Impacta també sobre les espècies natives directament ocupant l'espai i bloquejant el creixement d'arbustos mediterranis i el creixement de nous individus (Conser i Connor, 2009). *C. edulis* competeix amb les espècies natives pels pol·linitzadors (Moragues et al., 2005; Jakobsson et al., 2008). La invasió de *C. edulis* va relacionada amb una menor diversitat taxonòmica i funcional dels ecosistemes envaïts (Molinari et al., 2007; Jucker et al., 2013). Per tot això, *C. edulis* es troba inclosa dins del Catàleg espanyol d'espècies exòtiques invasores, i és per tant indispensable el disseny d'estratègies de gestió efectives per erradicar l'amenaça que suposa.

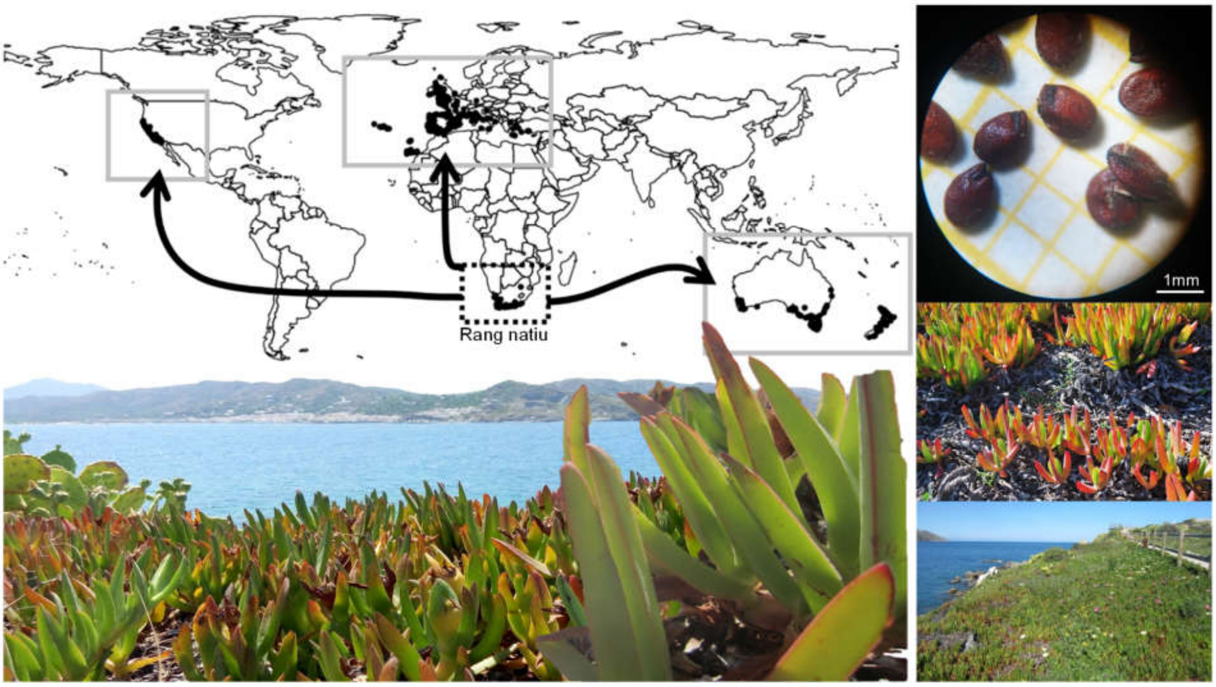

Figura 2. El model d'estudi, *Carpobrotus edulis*. Mapa global de presència (dades extretes de GBIF, 2019) i fotografies de l'espècie i les seves llavors d'individus del Cap de Creus.

Objectius

Objectius

L’objectiu principal d’aquesta tesi és entendre els mecanismes fisiològics que determinen l’èxit invasor de l’espècie *Carpobrotus edulis* al Mediterrani, relacionant les respostes a l’estrès, a la reproducció i a la senescència.

Per dur a terme aquest objectiu, es van proposar els objectius específics següents:

1) Contrastar la capacitat de fer front a les condicions ambientals del rang invasor de *C. edulis* amb una espècie nativa, a partir de l’avaluació de les respostes fotoprotectores davant l’estrès en condicions naturals del Mediterrani, especialment durant l’estiu i l’hivern.

2) Explorar la tolerància de *C. edulis* a l’estrès hídric durant l’estiu mediterrani, avaluant la influència de la càrrega reproductora durant aquest període.

3) Avaluar els mecanismes de resposta de *C. edulis* davant l’estrès per baixes temperatures de l’hivern mediterrani.

4) Determinar la importància de la reproducció per a la producció de bancs de llavors que determinen la capacitat de persistència de l’espècie al rang invasor.

5) Avaluar la diferenciació de *C. edulis* en el seu rang invasor en comparació del seu rang natiu per entendre el procés d’adaptació de l’espècie a un nou hàbitat i la seva expansió.

Informe del director

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El Dr. Sergi Munné Bosch, com a director de la Tesi Doctoral titulada "**Estrès, reproducció i senescència en l'èxit invasor: l'estudi de cas de *Carpobrotus edulis***" presentada per la doctoranda Erola Fenollosa Romaní,

INFORMA sobre el factor d'impacte i la participació de la doctoranda en cadascun dels articles inclosos en la memòria d'aquesta Tesi Doctoral

Capítol 1. Article "**Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum***", publicat a la revista *Physiologia Plantarum*, índex d'impacte (2018) de 3.000. En aquest treball es descriuen diversos mecanismes d'adaptació a l'estacionalitat típica del clima mediterrani de la planta invasora *Carpobrotus edulis*. La novetat del treball se centra en un estudi comparatiu de la planta invasora amb la planta nativa *Crithmum maritimum* amb un èmfasi especial en com els mecanismes de fotoprotecció contribueixen a la tolerància a l'estrès en ambdues espècies. La doctoranda ha realitzat tots els mostrejos, les anàlisis de les mostres i el tractament estadístic de les dades, i a més ha preparat totes les figures i ha redactat l'article, constant doncs com a primera autora del treball. La doctoranda ha demostrat una gran capacitat de treball, així com un excel·lent maneig en els mostrejos i una excel·lent predisposició en la introducció en l'ús de la cromatografia líquida d'alta resolució (HPLC) per a les anàlisis de molècules fotoprotectores i antioxidants (tocoferols i carotenoides). La doctoranda sobresurt en la capacitat d'anàlisi i interpretació dels resultats, així com en l'ús d'eines estadístiques i desenvolupament de nous models per explicar les dades, en aquest cas de plasticitat fenotípica.

Capítol 2. Article "**Environmental driven re-greening of senescing ramets in an invasive plant: occurrence, hormonal regulation and biological significance**", enviat a publicar a la revista *Plant Physiology and Biochemistry*, índex d'impacte (2018) de 3.404. En aquest treball es descriu per primera vegada l'existència del *re-greening* en fulles de *Carpobrotus edulis*, la seva importància en una millor comprensió de la possible reversibilitat de la senescència foliar i la seva regulació per citocinines, i com aquest *re-greening* de les fulles es podria considerar un mecanisme d'adaptació a l'estacionalitat típica del clima mediterrani en aquesta planta invasora. La novetat del treball recau sobretot en l'estudi de la possible reversibilitat de la senescència foliar i la seva regulació per citocinines, un aspecte de gran interès per la biologia bàsica, però que també té importància per comprendre millor l'ecofisiologia de les plantes invasores com *C. edulis*. La doctoranda ha realitzat tots els mostrejos, les anàlisis de les mostres i el tractament estadístic de les dades, i a més ha preparat totes les figures i ha redactat l'article, constant doncs com a primera autora del treball. La doctoranda ha demostrat una gran capacitat de treball, així com un excel·lent maneig en els mostrejos i en l'ús de l'HPLC, a més de mostrar també un bon domini de la cromatografia líquida acoblada a espectrometria de masses (LC-MS/MS), aquesta darrera tècnica emprada en aquest treball per a les anàlisis d'hormones. La doctoranda sobresurt en aquest treball en la seva capacitat d'anàlisi i interpretació dels resultats, així com en l'ús d'eines estadístiques per explicar les dades obtingudes, i finalment en la seva capacitat de lideratge, contrastada en aquest cas per ser l'autora de correspondència del treball.

Capítol 3. Article "**The nearest is not the closest: intraspecific variation in seed traits across diferent geographic scales may play a role in local adaptation of the invasive species *Carpobrotus edulis* to Mediterranean-type ecosystems**", enviat a publicar a la revista *Annals of Botany*, índex d'impacte (2018) de 3.454. En aquest treball es descriu per primera vegada la importància de la variabilitat a nivell intraespecific en llavors de *Carpobrotus edulis*, la seva importància en l'adaptació d'aquesta planta al clima mediterrani i les seves implicacions en la seva capacitat invasora en el litoral mediterrani. La novetat del treball recau sobretot en l'estudi de la variabilitat i la seva importància per comprendre millor l'ecofisiologia de les plantes invasores com *C. edulis*. El treball destaca per la importància de l'estudi de les llavors en una millor comprensió actual de les invasions biològiques, essent la biologia de les llavors un aspecte clau de la invasió que encara ha rebut poca atenció fins a l'actualitat degut a la dificultat de treballar en llavors a nivell experimental. La doctoranda ha realitzat tots els mostrejos, les anàlisis de les mostres i el tractament estadístic de les dades, i a més ha preparat totes les figures i ha redactat l'article, constant doncs com a primera autora del treball. La doctoranda ha demostrat una gran capacitat de treball, així com un excel·lent maneig en els mostrejos i en l'ús de diverses tècniques per a la caracterització de l'estat fisiològic de les llavors en poblacions naturals. La doctoranda sobresurt en aquest treball en la seva capacitat d'anàlisi i interpretació dels resultats, així com en l'ús d'un ampli ventall d'eines estadístiques per explicar les dades obtingudes, i finalment en la seva capacitat de lideratge, contrastada també en aquest cas per ser l'autora de correspondència del treball.

Capítol 4. Article "**Plasticity in the hormonal response to cold stress in the invasive plant *Carpobrotus edulis***", publicat a la revista *Journal of Plant Physiology*, índex d'impacte (2018) de 2.825. En aquest treball es descriu per primera vegada l'existència de plasticitat a nivell de la resposta hormonal de les fulles de plantes de *Carpobrotus edulis*, emfatitzant l'important paper que té la plasticitat fenotípica en l'adaptació d'aquesta espècie al clima mediterrani. La novetat de l'estudi recau sobretot en la combinació de tècniques bioquímiques per a les anàlisis del perfil hormonal amb aspectes més ecofisiològics de la resposta de les plantes invasores a situacions d'estrès abiòtic. Es descriu de forma original com diferents mecanismes de resposta hormonal poden portar a l'aclimatació a l'estrès en plantes com *C. edulis*, les quals mostren una gran plasticitat fenotípica i grau d'adaptació a condicions ambientals adverses. La doctoranda ha realitzat tots els mostrejos, les anàlisis de les mostres i el tractament estadístic de les dades, i a més ha preparat totes les figures i ha redactat l'article, constant doncs com a primera autora del treball. La doctoranda ha demostrat una gran capacitat de treball, així com un excel·lent maneig en els mostrejos i en l'ús de del LC-MS/MS per a les anàlisis del perfil hormonal, tècnica que la doctoranda demostra dominar a un excel·lent nivell. La doctoranda sobresurt en aquest treball en la seva gran capacitat de treball i habilitat per obtenir resultats de gran interès per l'avenç en l'estudi de l'ecofisiologia de les plantes invasores, no només a nivell ecològic sinó també a nivell bioquímic i de mecanismes d'acció hormonal.

Capítol 5. Article "**Increased chilling tolerance of the invasive species *Carpobrotus edulis* may explain its expansion across new territories**", publicat a la revista *Conservation Physiology*, índex d'impacte (2018) de 3.634. En aquest treball es descriu per primera vegada la importància de les condicions climàtiques en l'expansió de *Carpobrotus edulis* pel continent europeu, la seva importància en una millor comprensió del seu potencial invasor i la seva relació amb aspectes fisiològics. La novetat de l'estudi recau sobretot en la combinació d'eines fisiològiques amb la modelització per a comprendre millor aspectes claus de la invasió biològica. La doctoranda ha realitzat tots els mostrejos, les anàlisis de les mostres i el tractament estadístic de les dades, i a més ha preparat totes les figures i ha redactat l'article, constant doncs com a primera autora del treball. La doctoranda ha demostrat una gran capacitat de treball, així com un excel·lent maneig eines estadístiques i de modelització. La doctoranda sobresurt en aquest treball en la seva capacitat d'anàlisi matemàtica i interpretació dels resultats, així com en l'ús d'eines que permeten realitzar aproximacions realment interdisciplinars, i finalment en la seva capacitat de lideratge, contrastada en aquest cas per ser l'autora de correspondència del treball. La doctoranda demostra un excel·lent grau de maduresa científica durant tota la tesi doctoral arribant a un nivell extraordinari.

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Dr. Sergi Munné Bosch

Resultats

Capítol 1. Plasticitat fenotípica diferenciada en les estratègies fotoprotectores de l'espècie invasora *Carpobrotus edulis* i la nativa *Crithmum maritimum*

Chapter 1. Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum.*

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Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*

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Photoprotective strategies vary greatly within the plant kingdom and reflect a plant's physiological status and capacity to cope with environment variations. The plasticity and intensity of these responses may determine plant success. Invasive species are reported to show increased vigor to displace native species. Describing the mechanisms that confer such vigor is essential to understanding the success of invasive species. We performed an experiment whereby two species were monitored: *Carpobrotus edulis*, an aggressive invasive species in the Mediterranean basin, and *Crithmum maritimum*, a coexisting native species in the Cap de Creus Natural Park (NE Spain). We analyzed their photoprotective responses to seasonal environmental dynamics by comparing the capacity of the invader to respond to the local environmental stresses throughout the year. Our study analyses ecophysiological markers and photoprotective strategies to gain an insight into the success of invaders. We found that both species showed completely different but effective photoprotective strategies: in summer, *C. edulis* took special advantage of the xanthophyll cycle, whereas the success of *C. maritimum* in summer stemmed from morphological changes and alterations on β-carotene content. Winter also presented differences between the species, as the native showed reduced F_v/F_m ratios. Our experimental design allowed us to introduce a new approach to compare phenotypic plasticity: the integrated phenotypic plasticity index (PP_{int}), defined as the maximum Euclidian distance between phenotypes, using a combination of different variables to describe them. This index revealed significantly greater phenotypic plasticity in the invasive species compared to the native species.

As sessile organisms, plants must cope with local environmental conditions and be prepared to respond to stress periods. A typical response to most stresses is stomatal closure, which leads to suboptimal CO_2 assimilation rates, so photosynthetic demand may be exceeded by light absorption, i.e. photoinhibition (Takahashi and Badger 2011). If this excess of light is not avoided, it results in the formation of reactive oxygen species (ROS). If this increase in ROS is not counterbalanced by antioxidant defenses, it leads to photo-oxidative damage (Apel and Hirt 2004). Photoprotection and oxidative stress reflect the plant's physiological status and potential to

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Abbreviations – β-Car, β-carotene; Ax, antheraxanthin; Chl*a*/*b*, chlorophyll *a*/*b* ratio; DPS, de-epoxidation state of the xanthophyll cycle; ETR, electron transport rate; F_v/F_m, maximum quantum efficiency of photosystem II; LMV, leaf mass per volume; LHC, light-harvesting complexes; Lut, lutein; MDS, multidimensional scaling; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; PP_{int}, integrated phenotypic plasticity; VAZ, Zx + Ax + Vx; Vx, violaxanthin; Zx, zeaxanthin.

deal with environmental variations (Pintó-Marijuan and Munné-Bosch 2014) at multiple levels. First of all, light can be avoided by leaf or chloroplast movements and by light-screening processes by phenolic compounds (Takahashi and Badger 2011). Once light has reached the reaction centers, other photoprotective strategies take on importance. Carotenoids are a ubiquitous isoprenoid group of compounds within the plant kingdom (Esteban et al. 2009, Nisar et al. 2015) and play a dual role in the photosynthetic machinery, as they protect against photo-oxidative damage (Jahns and Holzwarth 2012) and act as accessory light-harvesting pigments (Yamamoto and Bassi 1996). This group is subdivided into carotenes, of which β-carotene (β-Car) is the most abundant, and xanthophylls, which contain oxygen and include lutein (Lut), violaxanthin (Vx), zeaxanthin (Zx), antheraxanthin (Ax) and neoxanthin. β-Car is especially efficient at scavenging the singlet oxygen ($^1O_2$) generated in photosystem II (PSII) from excited triplet chlorophyll (^{3}Chl*) as a consequence of excessive light (Ramel et al. 2012a). Lutein is the most abundant xanthophyll species in plants and is essential for protein folding and ^{3}Chl* quenching (Dall'Osto et al. 2006). Moreover, xanthophylls are crucial as physical quenchers that promote thermal dissipation or non-photochemical quenching (NPQ), an efficient energy-dissipation mechanism in plants (Demmig-Adams and Adams 1992, 1996). The de-epoxidation of Vx to Ax and Zx (components of the VAZ cycle) responds to different environmental stresses (Demmig-Adams and Adams 1996), and despite the fact that it represents an important cost (Raven 2011), it helps the plant survive during stress periods.

Plants have evolved to withstand the selection pressures of their habitat, and it is therefore generally assumed that a longer biogeographical history contributes to local adaptation (Kawecki and Ebert 2004). Compared to native species, invaders have a shorter adaptive history, but often perform better under environmental stress and thus displace natives (Simberloff et al. 2013).

Several hypotheses have been proposed to explain the main drivers of the invasion process (Catford et al. 2009). Some of these (e.g. evolution of increased competitive capacity, novel weapons and enemy release) assume that an invader's increased vigor compared to a native species determines its invasion success. The enemy release hypothesis, e.g. posits that the lack of natural enemies in the invasive range allows the invader to show such increased vigor (Blossey and Notzold 1995). Moreover, enhanced phenotypic plasticity has been hypothesized to allow species to become invasive and successfully colonize new habitats (Richards et al. 2006). However, the interpretation of phenotypic plasticity is not straightforward and depends on the comparison conditions or the experimental design (Hulme 2007). To understand the link between phenotypic plasticity and invasiveness, several approaches have been taken. Different plasticity indexes have also been developed, most of them using a single plant trait (Valladares et al. 2006), and it is common to present a mean phenotypic plasticity, based on the average of single plant traits plasticity (Peperkorn et al. 2005, Funk 2008, Matos et al. 2009, Grassein et al. 2010). A new approach that combines different traits could be a better estimator of phenotypic plasticity, as it contemplates the variation that exists across different areas of our knowledge of plants and that occurs between phenotypes, including possible trade-offs between variables (Meinzer 2003). Moreover, the use of ecophysiological markers in such calculations would contribute to understanding the success of invaders (Pintó-Marijuan and Munné-Bosch 2013).

We performed an experiment whereby two different species were monitored: *Carpobrotus edulis* as an invasive species and *Crithmum maritimum* as a native species. *C. edulis* is a monocarpic mat-forming succulent clonal plant that is native to South Africa. It is considered an aggressive invader in all Mediterranean climate regions, including California, Australia and the Mediterranean basin (D'Antonio and Mahall 1991). This species alters soil dynamics by creating a thick organic layer following its death, thus enhancing the growth of new generations (de la Peña et al. 2010, Novoa et al. 2013, Fenollosa et al. 2016) and inhibiting the germination of native species (Conser and Connor 2009, Novoa and González 2014). Furthermore, clonal traits such as division of labor and clonal integration have also been described as important mechanisms for the success of species invasion (Roiloa et al. 2010, 2013, 2016). As a monocarpic species, the reproductive ramets of *C. edulis* enter senescence after fruit formation, a process that may contribute to effective colonization (Fenollosa et al. 2016). Greater oxidative stress levels while reproductive ramets undergo senescence have been described (Zimmermann and Zentgraf 2005), and these could lead to clear physiological differences between reproductive and vegetative ramets. Moreover, *C. edulis* has been described as an inducible CAM species (Winter 1973, Earnshaw et al. 1987). The capacity to switch from C3 to CAM metabolism provides drought resistance mechanisms, which may enhance plant persistence under certain unfavorable environmental conditions. *C. maritimum* is a halophyte found on the rocky shores of the Mediterranean Sea and Atlantic Ocean (Ben Hamed et al. 2007, Ciccarelli et al. 2015). This plant has ethnopharmacological applications in folk medicine (essentially as an antiscorbutic and diuretic) due to its high levels of vitamin C, iodine, carotenoids and phenolic compounds (Ben Hamed et al. 2007, Jallali et al. 2014). Both species coexist in the Cap de Creus Natural Park (NE Spain), where we compared their annual response to the environmental conditions by measuring different physiological indicators. Our aim was to determine the capacity of *C. maritimum* and *C. edulis* to cope with local environmental variations throughout a year and evaluate the plasticity of their response.

Materials and methods

Study site and plant materials

Our study site was located in Far de s'Arenella (42°21′02.4″N 3°11′11.9″E) inside the protected area of the Cap de Creus Natural Park in Catalonia (NE Spain). Three zones in which the two species coexist were selected to ensure spatial representation (Appendix S1, Supplementary Information). Each zone measured approximately 25 m^2 and was separated from the others by at least 100 m. Climatic data (mean temperature, monthly precipitation and radiation) were provided by the *Servei Meteorològic de Catalunya* and recorded at the station closest to the study site, in Roses (42°16′01.8″N 3°13′26.1″E), between November 2014 and November 2015 (Appendix S3). In each zone, five individuals from each of the species *Carpobrotus edulis* and *Crithmum maritimum* (15 individuals per species) were selected randomly every two months between November 2014 and November 2015 for biochemical and ecophysiological measurements. On sunny days, four fully developed leaves per individual were taken at midday (11:00–13:00 solar time). One leaf was used for hydration (H), leaf mass per volume (LMV) and chlorophyll fluorescence measurements. The three remaining leaves were frozen in situ in liquid nitrogen and stored at −80°C until photosynthetic pigment quantification. In the summer, when the conditions were most stressful, the vegetative and reproductive ramets of *C. edulis* were differentiated. Flowering occurred between April and May, when the reproductive ramets of *C. edulis* underwent senescence.

Leaf water status and leaf mass volume

Hydration was measured as (FW−DW)/DW, where FW is the fresh weight and DW corresponds to the dry weight, obtained after drying the samples at 60°C until constant weight. LMV, expressed as g DW l^{-1}, was measured as an estimate of leaf succulence. Leaf volume was determined by fluid displacement.

Chlorophyll fluorescence

Measurements of chlorophyll fluorescence were performed with MiniPam (Heinz WalzGmbH, Effeltrich, Germany). The relative efficiency of PSII (Φ_{PSII}) was determined in situ, under photosynthetically active radiation (PAR) values of around 750 µmol m^{-2} s^{-1}. Φ_{PSII} was calculated as $(F_m'-F)/F_m'$, where F is the fluorescence yield of the light-adapted sample and F_m' is the maximum light-adapted fluorescence yield when a saturating light pulse of 800 ms duration (intensity approximately 3200 µmol m^{-2} s^{-1}) is superimposed on the prevailing environmental light levels (Oxborough and Baker 1997). The electron transport rate (ETR) was determined as ETR = Φ_{PSII} *0.5 *0.84 *PAR. After an hour of dark adaptation, the maximum quantum efficiency of PSII (F_v/F_m) was recorded. NPQ was calculated as $(F_m-F'_m)/(F'_m)$ (Oxborough and Baker 1997).

Pigment content

The content of photosynthetic pigments (chlorophylls and carotenoids) was measured by HPLC, as described by Munné-Bosch and Alegre (2000). Calcium carbonate was added to *C. maritimum* extracts as a buffering agent. De-epoxidation state of the xanthophyll cycle (DPS) was calculated as DPS = ((Zx + 0.5*Ax)/VAZ (Thayer and Björkman 1990). Chlorophylls are expressed by leaf surface, which was estimated by image analysis. In the case of *C. edulis*, whose leaves are isosceles in their transversal section, using two photographs per leaf to analyze the basal and lateral areas. This area was relative to the ground tissue used for the analysis.

Phenotypic plasticity

The plasticity of both species in response to the environmental conditions over the course of a year was measured by two different indexes. First, the phenotypic plasticity index (PPI) was calculated as described by Valladares et al. (2006) for each parameter analyzed: PPI = (maximum mean – minimum mean)/maximum mean. Second, we proposed the integrated phenotypic plasticity (PP_{int}), a new approach that considers an extensive series of traits that define a phenotype by using the relative distance between samples to estimate the maximum difference between phenotypes. Considering an *n*-dimensional space (where *n* is the number of variables), the relative position of each sample, obtained from a distance matrix, reveals the degree of dissimilarity between them. Therefore, the maximum distance between samples corresponds to the maximum capacity of a species (under the conditions considered) to alter

Table 1. Climatic conditions on the sampling days recorded at Roses. The measurements of mean temperature (T_m), relative humidity (HR), wind speed and global solar radiation (GSR 24 h) were provided by the *Servei Meteorològic de Catalunya*. Vapor pressure deficit (VPD) was calculated as described by Buck (1981).

	Month	Day	T_m (°C)	HR (%)	Wind speed (m s^{-1})	GSR 24 h (MJ m^{-2})	VPD (Pa)
2014	November	13/11	11.8	82	1	10.5	249.0
2015	January	25/01	10.8	32	11.2	11.0	749.1
	March	01/04	14.8	46	5.6	22.4	908.5
	May	04/06	24.1	48	1.7	29.5	1560.5
	July	16/07	26.2	66	2.9	27.7	1156.2
	September	25/09	20.6	49	4.1	19.4	1236.9
	November	30/11	8.7	76	0.9	9.1	269.8

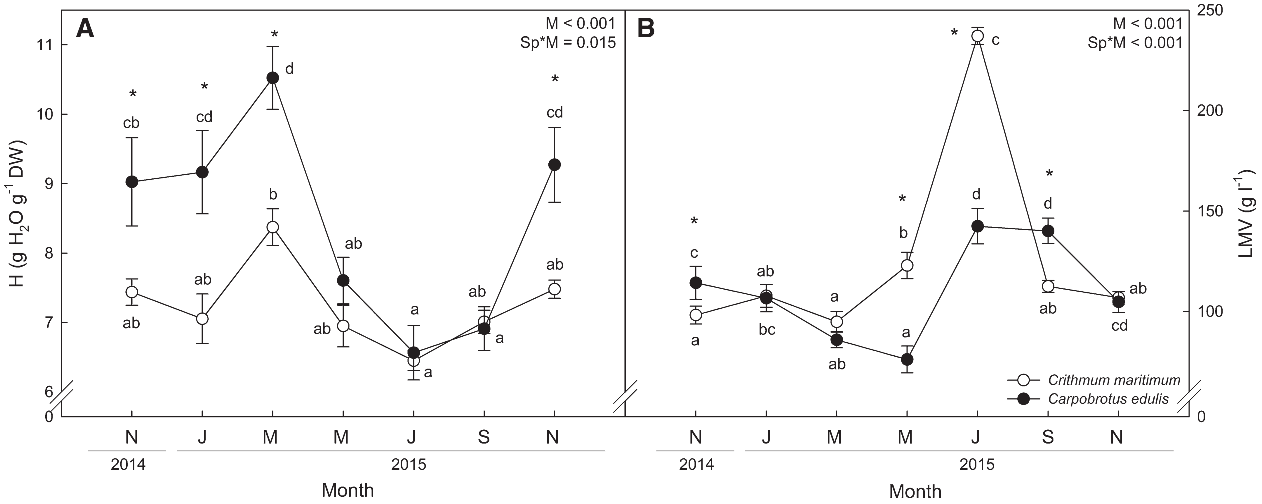

Fig. 1. Water content and leaf morphology index of the two species studied (*Carpobrotus edulis* and *Crithmum maritimum*) from November 2014 to November 2015. Hydration (A) and leaf mass per volume (B). Letters (a, b, c, d) show significant differences between time in each species and asterisks (*) indicate significant differences between species within each month. *P* values from the significant effects of the general model are shown on each plot. All the *P* values are shown in Appendix S2 (Supplementary information).

all the different measured variables that describe a phenotype. This approximation considers the fact that some traits are subjected to trade-offs and considers the plasticity of a phenotype, not single traits. In our study, we considered all the measured parameters for each species to calculate the Euclidian distance between samples and centroids in the different months studied. After identification of the furthest centroids (which represent the most different monthly phenotypes), we recalculated the distance between centroids when replacing, one by one, each of the samples under study to describe the centroid distance variability. All variables were standardized by considering all data before analysis. Multidimensional scaling (MDS) was performed to visualize, in reduced dimensions, the distances between the samples and the relative positions of the data for different months. To confirm that the most different phenotypes identified were statistically different, we performed permutational ANOVA (PERMANOVA) within the different distances between the monthly samples in the distance matrix.

Statistics

A three-way mixed ANOVA was performed to test the significance of the fixed factors 'Species' and 'Month' and the random factor 'Zone'. For the chlorophyll fluorescence measurements taken under sunlight, PAR was used as a covariable. The Bonferroni adjustment was used to perform pairwise comparisons. Differences were considered significant at $P < 0.05$. Normality and homoscedasticity were tested trough the Kolmogorov–Smirnov and Levene tests, respectively. All variables met the ANOVA requirements. Statistical analysis was performed with SPSS 11.5 for Windows statistical software package. The distance analysis, PERMANOVA and MDS were performed with the Permanova + package in Primer-E. Data are always represented as mean ± SE (n = 15).

Results

Plasticity of photoprotection in the invasive species vs the native species

The period analyzed typically represented the Mediterranean climate, with warm, dry summers (Appendix S3). The maximum temperature reached during the period under study was 38.2°C in July, and the minimum temperature was 5.7°C in February. The climatic conditions on each sampling day are shown in Table 1. *Carpobrotus edulis* and *Crithmum maritimum* differed in their responses to the same environmental conditions and the greatest differences arose under the most stressful conditions, i.e. in summer. No significant effects were found for the 'Zone' random factor in any of the variables considered (Appendix S2). Both species widely modulated their water content during the year, from 4 to 14.5 g H_2O g^{-1} DW (Fig. 1A). *C. edulis* maintained a higher level of hydration in autumn, winter and spring compared to *C. maritimum*, but there were no significant differences between species during the summer, when both species showed their lowest hydration values. Some changes in leaf morphology occurred during the year, as a sudden increase in the LMV in July for *C. maritimum*, which nearly doubled its winter values (Fig. 1B). Similarly, an increase in LMV was found for *C. edulis* in the summer, despite the fact that this increase was only approximately 30%. Both species showed a strong negative correlation between LMV and H (Appendices S4 and S5), thus indicating the dependence of leaf morphology on water content.

Photosynthetic mechanisms showed different strategies to cope with the changing climatic conditions. Significant differences in the ETR between species were found almost every month except for March and November (Fig. 2A). *C. maritimum* always maintained higher values, from approximately 99.22 ± 7.24 µmol e^- m^{-2} s^{-1} in winter to 160.17 ± 5.26 µmol e^- m^{-2} s^{-1} in summer. It is noteworthy that *C. edulis* did not increase its ETR in the summer in the same way *C. maritimum* did; in fact, the lowest ETR values for this species were measured during the summer months. A significant positive correlation was found between ETR and daily radiation *in C. maritimum* (Fig. 3), but not in *C. edulis*. Some *C. edulis* and *C. maritimum* individuals suffered photoinhibition, as their F_v/F_m fell below 0.75 at different months (Fig. 2B): *C. maritimum* showed the lowest F_v/F_m values in January, while the lowest values for *C. edulis* occurred in July. The species also differed in their use of the thermal quenching, as shown by the NPQ values (Fig. 2C). *C. edulis* individuals took advantage of this path to dissipate the excess light (especially during the summer months), and reached values of 5.64 ± 0.67 in July; however, *C. maritimum* maintained lower, more constant NPQ values throughout the year, from 0.85 ± 0.07 in September to 2.47 ± 0.26 in March.

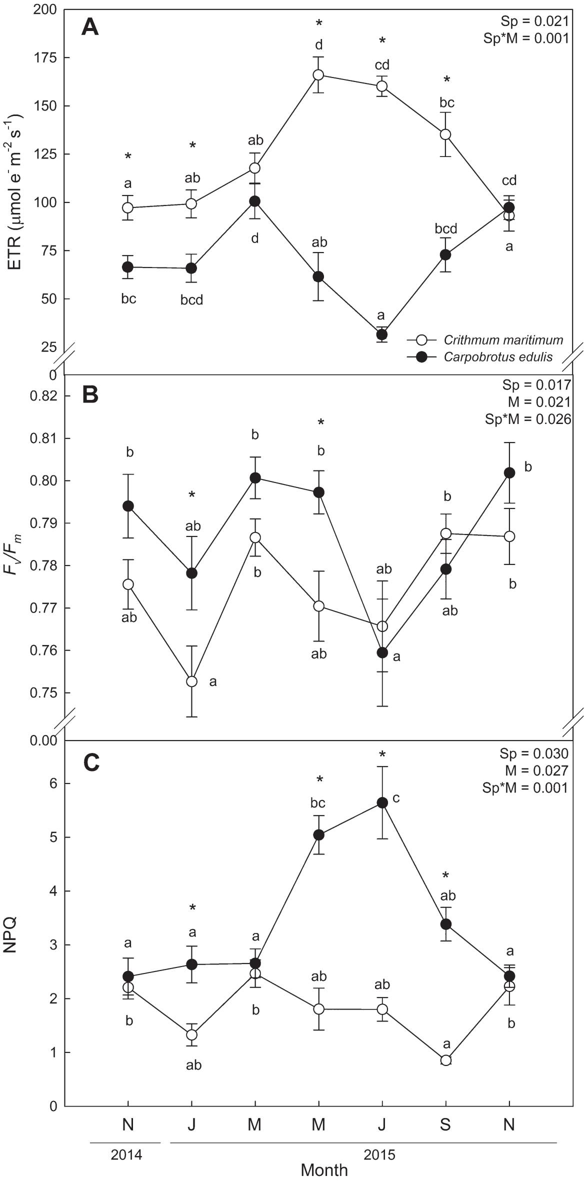

Fig. 2. Chlorophyll fluorescence parameters of the two species studied (*Carpobrotus edulis* and *Crithmum maritimum*) from November 2014 to November 2015. (A) Electron transport rate (ETR), (B) maximum quantum efficiency of photosystem II (F_v/F_m) and (C) non-photochemical quenching (NPQ). Letters (a, b, c, d) show significant differences between time in each species and asterisks (*) indicate significant differences between species within each month. *P* values from the significant effects of the general model are shown on each plot. All the *P* values are shown in Appendix S2 (Supplementary information).

Both species responded to the summer climatic conditions with changes in the composition of light-harvesting complexes (LHC), as alterations in the total chlorophyll

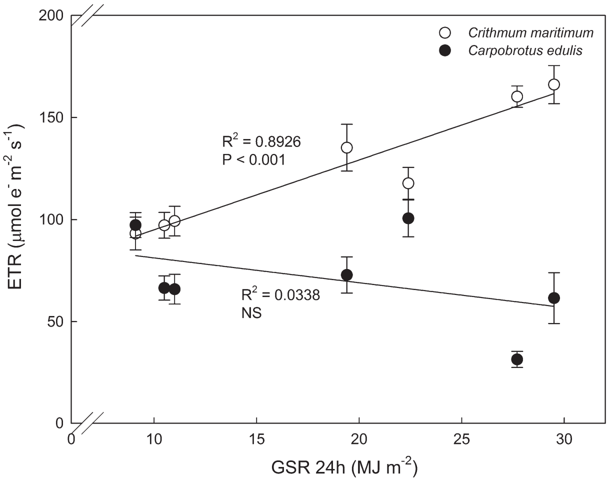

Fig. 3. The electron transport rate (ETR) response from *Carpobrotus edulis* and *Crithmum maritimum* to the daily global radiation (GSR 24 h) of the sampling day, including the seven months analyzed between November 2014 and November 2015.

content (Chl_T), the chlorophyll *a*/*b* ratio (Chl*a*/*b*) and the different carotenoids were observed (Fig. 4). Both *C. edulis* and *C. maritimum* showed a reduction in Chl_T in summer, especially the invasive species, which presented a significant reduction, from 263.2 ± 23.0 (November 2015) to 161.0 ± 10.0 mmol m^{-2} (July) (Fig. 4A). Despite this common reduction, the Chl*a*/*b* ratio changed differentially between species (Fig. 4B). *C. maritimum* gradually increased the ratio from winter to summer, whilst *C. edulis* showed the lowest ratio during the summer months, 2.81 ± 0.09, compared to 3.51 ± 0.05 in *C. maritimum* in July. *C. maritimum* presented a lower xanthophyll content (around 200 mmol VAZ mol^{-1} Chl_T all year, compared to the variation of *C. edulis* from 332.98 ± 26.14 to 819.19 ± 40.30 mmol VAZ mol^{-1} Chl_T), but a significant increase was found between May and January (Fig. 4C). On the other hand, the VAZ content of *C. edulis* increased significantly in summer (May to September). *C. edulis* showed a higher proportion of Ax and Zx with respect to Vx, as the DPS was higher than that of the native species throughout the year (Fig. 4D). VAZ was strongly correlated with DPS (Appendix S4 and S5), i.e. the more xanthophylls present, the more were converted to Zx and Ax. In summer, a substantial increase in DPS occurred in *C. edulis* (from 0.54 ± 0.03 in November 2015 to 0.78 ± 0.02 in July). The increased need to dissipate energy was negatively correlated with ETR in the case of *C. edulis,* thus indicating the high costs invested in photoprotection (Appendices S4 and S5). The lutein content responded similarly to VAZ in *C. edulis*, as shown by the significant correlation between these two variables (Appendix S4), thus highlighting the role of xanthophylls in the photoprotection of this species. While the invasive species showed a gradual increase in Lut content from winter to summer, no significant differences were found in *C. maritimum* between the different months (Fig. 4E). Finally, the species showed mirror symmetry in *β*-Car content over the course of the year (Fig. 4F): *C. maritimum* maintained lower values from winter to spring and experienced a strong increase in July and September (approximately 50%), while *C. edulis* showed a significant reduction (approximately −60%).

Vegetative vs reproductive ramets of *C. edulis*

In summer, under the most stressful conditions, we differentiated between the vegetative and reproductive ramets of *Carpobrotus edulis*, whose photosynthetic performance and pigment content showed different trends (Tables 2 and 3). No differences were detected in leaf morphology (LMV) between ramet types, but the reproductive ramets maintained hydration values that were approximately 10% higher (Table 2). Moreover, no differences between ramet types were observed in F_v/F_m, but the vegetative ramets maintained higher ETR throughout the three summer months (Table 2). In July, the differences in ETR and hydration between reproductive and vegetative ramets seemed to be minimal, but there were clearer differences in thermal dissipation. Also in July, the NPQ of the reproductive ramets reached values of 7.65 ± 1.10, the highest levels recorded in this experiment. Moreover, DPS was higher in reproductive ramets (Table 3), which, together with a higher NPQ, suggests that they make greater use of the VAZ cycle to dissipate excessive energy (Fig. 5). Significantly lower Chl_T and higher xanthophyll levels (VAZ and Lut) were found in reproductive ramets (Table 3), thus indicating that the photosynthetic pigments play different roles in the senescence process.

Phenotypic plasticity quantification

C. edulis showed higher phenotypic plasticity than the coexisting native species *C. maritimum*. When all of the measured variables are taken into account, the invasive species showed a mean phenotypic plasticity (based on the PPI) of 0.415 ± 0.05, and that of the native species was 0.399 ± 0.05. However, this difference was not significant (*P* value = 0.325). This mean plasticity was clearly influenced by the variables considered and their variability. Despite a lower PPI mean, the native species presented a slightly higher PPI in LMV, F_v/F_m NPQ, DPS, Vx, VAZ and *β*-Car (Fig. 6). The integrated phenotypic plasticity index (PP_{int}) also demonstrated

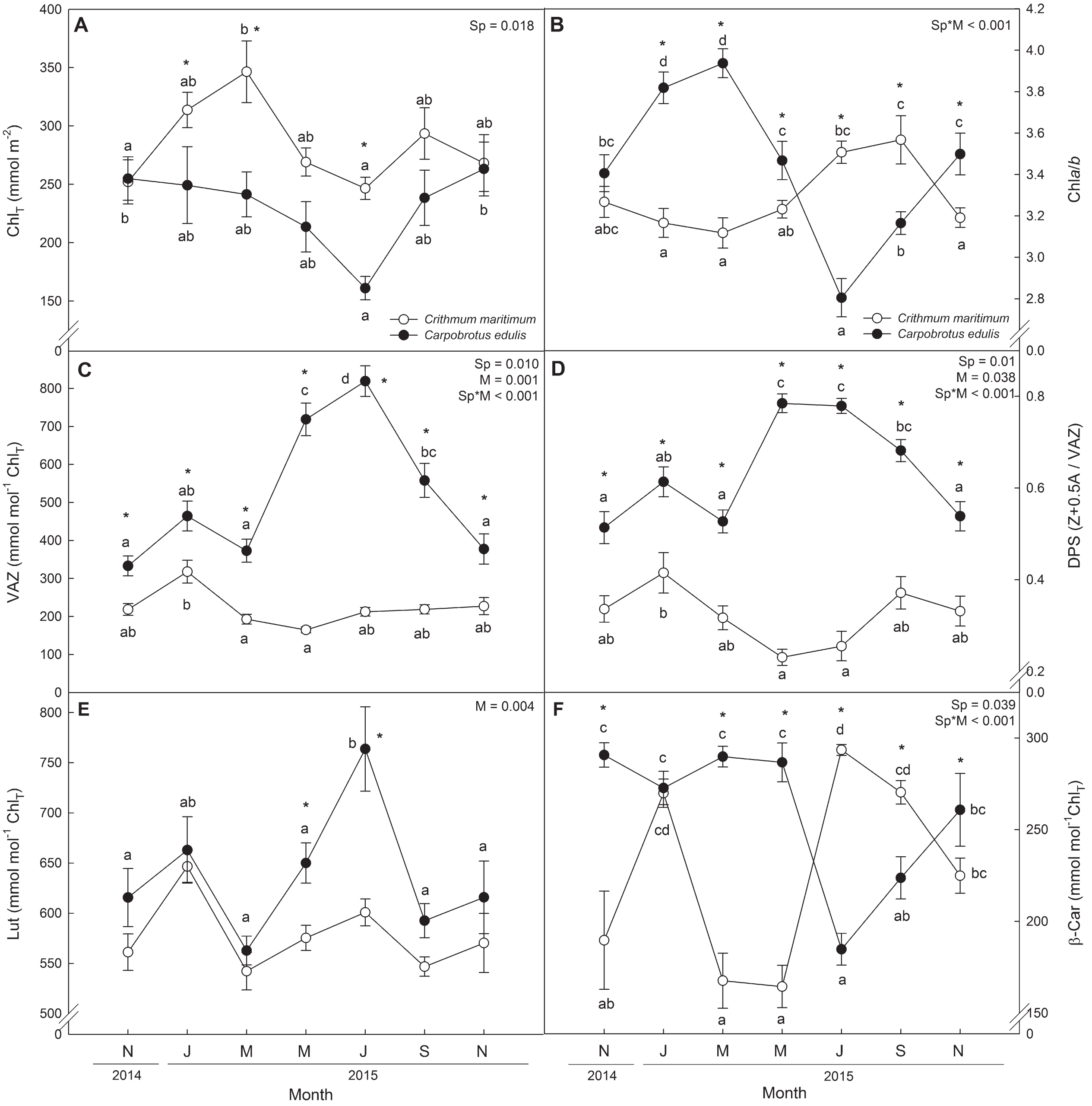

Fig. 4. Photosynthetic pigment composition of the two species studied (*C. edulis* and *C. maritimum*) from November 2014 to November 2015. Total chlorophyll content (Chl_T) (A), Chl*a*/*b* ratio (B), total xanthophyll content (VAZ) (C), de-epoxidation state (DPS) (D), lutein (E) and β-carotene (F). Letters (a, b, c, d) show significant differences between time in each species and asterisks (*) indicate significant differences between species within each month. *P* values from the significant effects of the general model are shown on each plot. All the *P* values are shown in Appendix S2 (Supplementary Information).

higher plasticity in the invader. The maximum distance between phenotypes was significantly different (*P* value < 0.001) between species: 1.155 ± 0.003 in *C. edulis* and 0.771 ± 0.004 in *C. maritimum*. The more distant phenotypes in the invasive species occurred between November 2015 and July, and in the native between March and July (Table 4). PERMANOVA analysis revealed that in both cases the different monthly phenotypes were statistically different (*P* value < 0.001 in both cases). All the *P* values between months for the two species are shown in Appendix S7.

The multidimensional scaling analysis represented the relative distance between samples in a

Table 2. Water content, leaf morphology index and chlorophyll fluorescence parameters of the reproductive (Rep) and vegetative (Veg) ramets of *Carpobrotus edulis* in May, July and September 2015. ETR, electron transport rate; F_v/F_m, maximum quantum efficiency; H, Hydration; LMV, leaf mass volume; NPQ, non-photochemical quenching. *Significant differences (P value < 0.05) between ramet type in each month.

	H (g H_2O g^{-1} DW)		LMV (g l^{-1})		ETR (µmol e^- m^{-2} s^{-1})		F_v/F_m		NPQ	
Month	Veg	Rep	Veg	Rep	Veg	Rep	Veg	Rep	Veg	Rep
May	7.60 ± 0.34	8.32 ± 0.44	76.14 ± 6.70	78.87 ± 7.24	61.53 ± 12.45*	36.99 ± 6.14*	0.80 ± 0.01*	0.77 ± 0.01*	5.04 ± 0.36	5.36 ± 0.23
July	6.56 ± 0.39	7.06 ± 0.40	142.46 ± 8.76	128.43 ± 5.72	31.56 ± 3.87	20.15 ± 3.05	0.76 ± 0.01	0.77 ± 0.01	5.64 ± 0.67*	7.65 ± 1.10*
September	6.91 ± 0.32*	7.80 ± 0.26*	140.11 ± 6.38	118.70 ± 4.07	72.84 ± 8.85*	35.23 ± 2.54*	0.78 ± 0.01	0.76 ± 0.01	3.38 ± 0.31	4.11 ± 0.26

reduced-dimension scenario, and provides an idea of the data distribution across the different months. In the case of *C. edulis* (Appendix S6A), there is a slight gradient from the phenotypes from July to November through the samples from other months. In *C. maritimum* the data seem to be distributed in three different directions (Appendix S6B). Although the more distant phenotypes occurred between July and March, January also showed relatively similar distance values against those two months (Table 4) and was statistically different from July and March (P value < 0.001 in both cases). To understand and identify the main drivers of the differences between samples, defining the principal components (through principal component analysis, PCA) and identifying the strongest coefficients for the different original variables may prove very helpful. However, visualizing the different variables within the MDS is preferable (Appendix S6), as this analysis takes the whole data set into account, while less important variables are omitted in PCA (up to the percentage of variation explained by the main components).

Discussion

Different, but not inferior, photoprotective strategies

In this study, *Carpobrotus edulis,* an aggressive invader that represents an important threat to the biodiversity of the Mediterranean coast (Vilà et al. 2004), presented a completely different photoprotective strategy from a native species (*Crithmum maritimum*) to cope with variations in the climatic conditions throughout the year. Drought stress in summer is a common adversity on the Mediterranean coast, and species have therefore developed different strategies to respond to the water shortage, warm temperatures and high solar radiation (Zunzunegui et al. 2011). Previous studies have evaluated the role of photoprotection in Mediterranean species to cope with summer conditions (Munné-Bosch and Alegre 2000, Munné-Bosch and Peñuelas 2004, Baraldi et al. 2008), and all of them have highlighted the efficiency of carotenoids to overcome the excessive light received. Summer clearly affected *C. edulis* and *C. maritimum*, as shown by the reduction in hydration during those months. Moreover, they both experienced photoinhibition, as F_v/F_m values were lower than 0.75 (Kalaji et al. 2014). In response to such harsh environmental conditions, *C. edulis* took advantage of the xanthophyll cycle to cope with the energy excess, with a two-fold increase in the VAZ xanthophyll pool during the summer months. This response is known to occur in a wide range of plant species in response to excessive light and other stresses (Demmig-Adams and Adams 1992, 1996). In addition to the xanthophyll cycle, Lut also seemed to play an important role in the photoprotective strategy of *C. edulis*. Lutein antioxidant activity, described by Dall'Osto et al. (2006), may explain its increase in *C. edulis* in July. Besides the summer-triggered xanthophyll response, the chlorophyll composition also played an important role in the photoprotective response. Antenna size regulation is a well-known response to different stresses (Morosinotto and Bassi 2012), and a lower Chl*a*/*b* ratio is associated with a reaction center reduction (Evans 1988), as Chl*a* is located in both LHCs and reaction centers, whereas Chl*b* is mainly restricted to LHCs (Croce and Van Amerongen 2011). Chlorophyll loss of approximately 50% has been reported under severe stress conditions in water-stress experiments (using plants with a relative water content of approximately 68%) (Munné-Bosch and Peñuelas 2004). Thus, the photoprotection strategy of *C. edulis* seemed to contrast with the responses of *C. maritimum*, as the Chl_T in *C. maritimum* remained stable throughout the whole year. ETR and ETR-daily radiation correlation (Fig. 3) also revealed very different responses in *C. maritimum* compared to *C. edulis*: the former showed maximum ETR values during the more stressful months, since they responded linearly to radiation, i.e. they were only limited by the amount of light received and did not show signs of photosynthetic machinery saturation; the latter responded to the increase in radiation with a reduction in ETR and by unfurling its photoprotective mechanisms. The fact that *C. maritimum* maintained

Table 3. Photosynthetic pigment composition of the reproductive (Rep) and vegetative (Veg) ramets of *Carpobrotus edulis* in May, July and September 2015. DPS, de-epoxidation state; Chl_T, total chlorophyll content; VAZ, xanthophyll content. *Significant differences (*P* value < 0.05) between ramet type in each month.

Month	Chl_T (mmol m^{-2})		Chl*a*/*b*		VAZ (mmol mol^{-1} Chl_T)		DPS		Lut (mmol mol^{-1} Chl_T)		β-Car (mmol mol^{-1} Chl_T)	
	Veg	Rep	Veg	Rep	Veg	Rep	Veg	Rep	Veg	Rep	Veg	Rep
May	196.13 ± 13.55*	118.57 ± 17.43*	3.47 ± 0.09*	3.26 ± 0.07*	688.91 ± 44.20	770.21 ± 50.80	0.78 ± 0.02	0.79 ± 0.02	633.26 ± 15.69	740.41 ± 49.30	292.74 ± 11.26*	251.18 ± 11.81*
July	161.05 ± 10.00*	72.97 ± 8.11*	2.81 ± 0.09	2.72 ± 0.06	813.89 ± 42.91*	1222.37 ± 98.45*	0.78 ± 0.02	0.81 ± 0.01	763.74 ± 42.09*	1246.66 ± 118.33*	184.81 ± 8.63	209.08 ± 18.09
Sept.	272.75 ± 26.4*	131.43 ± 17.90*	3.17 ± 0.05	3.25 ± 0.10	557.69 ± 44.45	651.41 ± 52.25	0.68 ± 0.02*	0.74 ± 0.02*	587.57 ± 17.35	635.02 ± 29.52	223.74 ± 11.52	218.18 ± 7.03

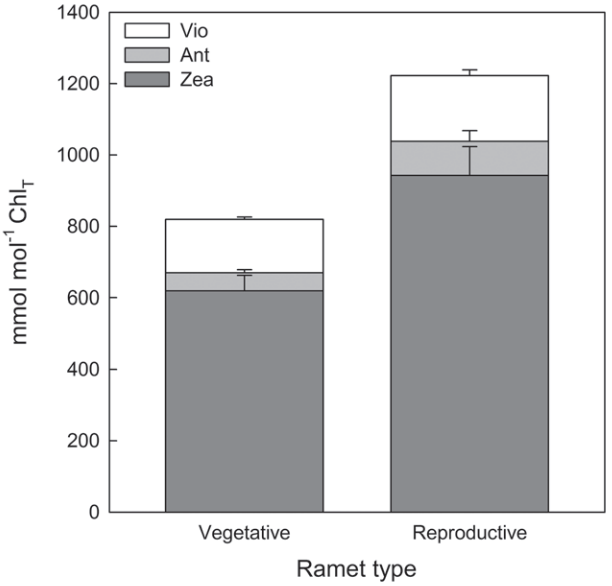

Fig. 5. Total xanthophyll composition (violaxanthin, antheraxanthin and zeaxanthin) in vegetative and reproductive ramets of *C. edulis* in July 2015.

stable chlorophyll levels and higher ETR values under the same levels of daily radiation could be attributed to alternative photoprotective mechanisms. Despite the higher radiation in July, the native did not increase its DPS during the summer months. Instead, *C. maritimum* increased its β-Car levels in response to the water content reduction, as revealed by the negative correlation with hydration. The antioxidant role of β-Car is involved in eliminating singlet oxygen ($^1O_2$) (Ramel et al. 2012a), which is produced under excessive light in PSII. However, under severe stress, β-Car may be rapidly transformed to β-cyclocitral and other oxidation products, which may act as stress-signaling molecules (Ramel et al. 2012b, Havaux 2014). The β-Car reduction in *C. edulis* may be explained by the higher oxidative stress levels compared to *C. maritimum* as a result of dehydration (as shown by the correlation between β-Car and H) and the consequent photoinhibition (as shown by the correlation between β-Car and F_v/F_m). In addition to β-Car content modulation, alterations in leaf morphology (as shown by LMV dynamics throughout the year) may play a role in the photoprotective strategy of *C. maritimum* by diminishing the amount of radiation absorbed during the summer. However, other photoprotective mechanisms could be involved, including paraheliotropism (Pastenes et al. 2005), chloroplast movement and an increase in the epidermal phenolic compounds responsible for screening solar radiation (Takahashi and Badger 2011). Previous studies with this native species have shown that plants at the reproductive stage (August) are richer in phenolic

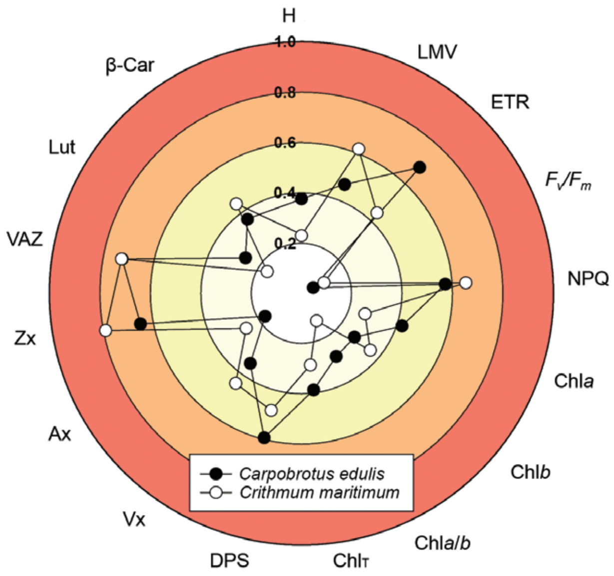

Fig. 6. Phenotypic plasticity index (PPI) in each trait for the two species studied: *Carpobrotus edulis* and *Crithmum maritimum*. Ax, antheraxanthin; *β*-Car, *β*-carotene; Chl*a*, chlorophyll *a;* Chl*b*, chlorophyll *b;* Chl*a*/*b*, Chl*a*/*b* ratio; Chl_T, Chl*a* + Chl*b*; DPS, de-epoxidation state; ETR, electron transport rate; F_v/F_m, maximum quantum efficiency of photosystem II; H, hydration; LMV, leaf mass per volume; Lut, lutein; NPQ, non-photochemical quenching; VAZ, Zx + Ax + Vx; Vx, violaxanthin; Zx, zeaxanthin.

compounds than plants at the vegetative stage (June), and consequently exhibit higher antioxidant activity (Jallali et al. 2012). Phenolic compounds, in which *C. maritimum* is richer, could help overcome the stressful conditions of summer (Meot-Duros and Magné 2009, Jallali et al. 2012, 2014). *C. edulis* does not respond with morphological alterations and may receive a higher amount of total solar energy. Therefore, greater photoprotection investment at chloroplast level was needed, but this was detrimental to photosynthetic efficiency (i.e. ETR reduction).

Winter oxidative stress in *Crithmum maritimum*

Despite the fact that summer was the key period in the dynamics analyzed, some differences between the native and invasive species were also detected in winter. Photoinhibition appeared in some individuals of *C. maritimum* in January. Winter photoinhibition is a known phenomenon in some Mediterranean species, as a consequence of the low temperatures, short photoperiods and clear days with elevated irradiance (Oliveira and Peñuelas 2004, Ciccarelli et al. 2015, Morales et al. 2016). A slight decrease in F_v/F_m in winter has also recently been described in *C. maritimum*, despite the fact that summer photoinhibition was clearly stronger (Ciccarelli et al. 2015). Winter in overwintering evergreens is known to cause the photoprotective mechanisms to unfurl, especially the xanthophyll cycle (Adams et al. 2004, Míguez et al. 2015). In our study case, *C. maritimum* responded to winter photoinhibition (during which it experienced the lowest annual F_v/F_m values) with an increase in the xanthophyll pool size (VAZ) and *β*-Car levels, as well as the highest DPS annual values. In all the studies included in a meta-analysis by Míguez et al. (2015), stronger winter photoinhibition (measured as the relative decrease in F_v/F_m) was always accompanied by higher DPS, but none of them described the role of *β*-Car in winter photoinhibition. As Morales et al. (2016) already found in the Mediterranean dwarf palm (*Chamaerops humilis*), both Lut and *β*-Car could be implicated in the photoprotection mechanisms during the lowest F_v/F_m as antioxidants to cope with the ROS generated due to excessive light (Dall'Osto et al. 2006, Triantaphylidès and Havaux 2009, Ramel et al. 2012a, 2013). The double *β*-Car peak (in January and July) found in *C. maritimum* suggested that comparable oxidative stress occurred during those periods. *β*-Car could therefore play a crucial role in the alleviation of winter oxidative stress in *C. maritimum*.

Table 4. Average Euclidian distance between/within months, considering all of the variables analyzed for the two species under study: *Carpobrotus edulis* and *Crithmum maritimum*[a]. [a]The maximum Euclidian distance, i.e. the integrated phenotypic plasticity (PP_{int}), is shown in bold.

	Carpobrotus edulis							*Crithmum maritimum*						
	Nov 15	Jan	March	May	Jul	Sept	Nov 14	Nov 15	Jan	March	May	Jul	Sept	Nov 14
Nov 15	0.594							0.578						
Jan	0.710	0.774						0.632	0.532					
March	0.649	0.694	0.540					0.605	0.673	0.540				
May	0.943	0.890	0.950	0.686				0.637	0.742	0.595	0.397			
Jul	1.113	1.040	1.140	0.787	0.721			0.735	0.720	**0.771**	0.607	0.400		
Sept	0.763	0.776	0.756	0.778	0.853	0.660		0.631	0.624	0.650	0.638	0.656	0.581	
Nov 14	0.621	0.723	0.608	0.966	**1.155**	0.765	0.623	0.589	0.624	0.588	0.665	0.736	0.626	0.617

Photoprotection during ramet senescence

C. edulis is a perennial plant species with both sexual and vegetative propagation. It reproduces from May to June, but flowering only occurs in some ramets, which undergo senescence (Fenollosa et al. 2016). At ramet level, *C. edulis* is a monocarpic plant. Senescence in monocarpic plants is invoked to mobilize enough nutrients for seeds to ensure their survival (Davies and Gan 2012, Thomas 2013). Chloroplasts are one of the target organelles once senescence is induced, and this leads to chlorophyll degradation, a decrease in photosynthesis and ROS accumulation (Wingler et al. 2004). Differentiating between the reproductive and vegetative ramets of *C. edulis* after flowering allowed us to observe the role of photoprotection in the species during plant senescence. Photoprotection and antioxidants play an important role during ramet senescence by controlling ROS formation in both space and time (Munné-Bosch et al. 2001). During the remobilization phase, increased photoprotection and antioxidant content (xanthophyll content, DPS, α-tocopherol, β-Car and antioxidant enzymes) have been widely described, whereas these levels may decrease during the terminal phase of senescence (Juvany et al. 2013). In our experiment, one of the clearest differences between the reproductive and vegetative ramets of *C. edulis* was Chl_T. Chlorophyll degradation is part of the senescence process and allows nutrients to be recycled (Munné-Bosch and Alegre 2004). Reproductive ramets showed higher dissipation needs than vegetative ramets. Other studies have indicated that the increased DPS during plant senescence is due to a decrease in the Vx levels rather than an increase in Zx or Ax (Munné-Bosch and Peñuelas 2003). However, the higher DPS in the reproductive ramets was due to both higher Zx and Ax levels. In fact, the photoprotective responses of reproductive ramets seemed to be more stress sensitive compared to the vegetative ramets. DPS, VAZ and Lut levels, which were higher during the three months analyzed, showed a peak concentration in July, the most stressful month. Reproductive ramets may have more photoprotection and may respond more severely to the same change in environmental conditions compared to vegetative ramets, perhaps because of the delicate oxidative stress control during the senescence process.

Despite the higher thermal dissipation needs, reproductive ramets presented higher hydration values. This may be due to protein degradation during the senescence process, a fact that inhibits photosynthesis and, in turn, promotes stomatal closure, thus maintaining a higher water content (Thimann and Satler 1979). As an inducible CAM species, *C. edulis* may be especially efficient at triggering stomatal closure responses. Facultative CAM has been the subject of numerous studies, which have focused on the mechanisms that trigger the CAM shift (Winter and Holtum 2014) and have shown, for example, that *Kalanchoe blossfeldiana* leaves develop CAM as they age (Queiroz and Brulfert 1982) as part of a development program. However, to our knowledge, no studies have evaluated the activation of CAM metabolism during leaf senescence in CAM-facultative plant species.

New approaches to phenotypic plasticity

The integrated phenotypic plasticity index allowed us to estimate the capacity of each species to alter its whole physiological status in response to the environmental conditions and revealed that *C. edulis* was more plastic than the coexisting native *C. maritimum*. The differences in the environmental conditions between summer and winter determined the production of the most different phenotypes in both species, in spite of the fact that the native species presented three different genotypes, which may correspond to adaptation to summer stress (July), non-stressful conditions (March) and cold stress (January).

The PP_{int} could be used as a new approach to help understand phenotypic plasticity, but it has two primary requirements: (1) the comparison must be made under the same environmental variations and (2) reversible variables, i.e. those that have no direction with time and ensure plant survival, must be used. In Fig. 7, we summarize some simple variables that can be used when calculating the PP_{int} to ensure a complete phenotype description. Moreover, this overview includes variables from the characterization of a number of prospective plant species (fitness, morphological and physiological characteristics), which may contribute to a complete phenotype description. To visualize the spatial distribution of the relative distance between samples, MDS is recommended. The PP_{int} is recommended as a new approach to understanding phenotypic plasticity and comparing species.

Advantages of greater phenotypic plasticity

Several hypotheses have been proposed to explain the increased vigor of invaders (Catford et al. 2009). A jack-of-all-trades strategy (or species with a 'general purpose genotype', as proposed by Baker (1965)) emphasizes the role of phenotypic plasticity in invasion success (Richards et al. 2006), but a master-of-some strategy highlights the importance of local adaptation (Parker et al. 2003). Moreover, a combined strategy

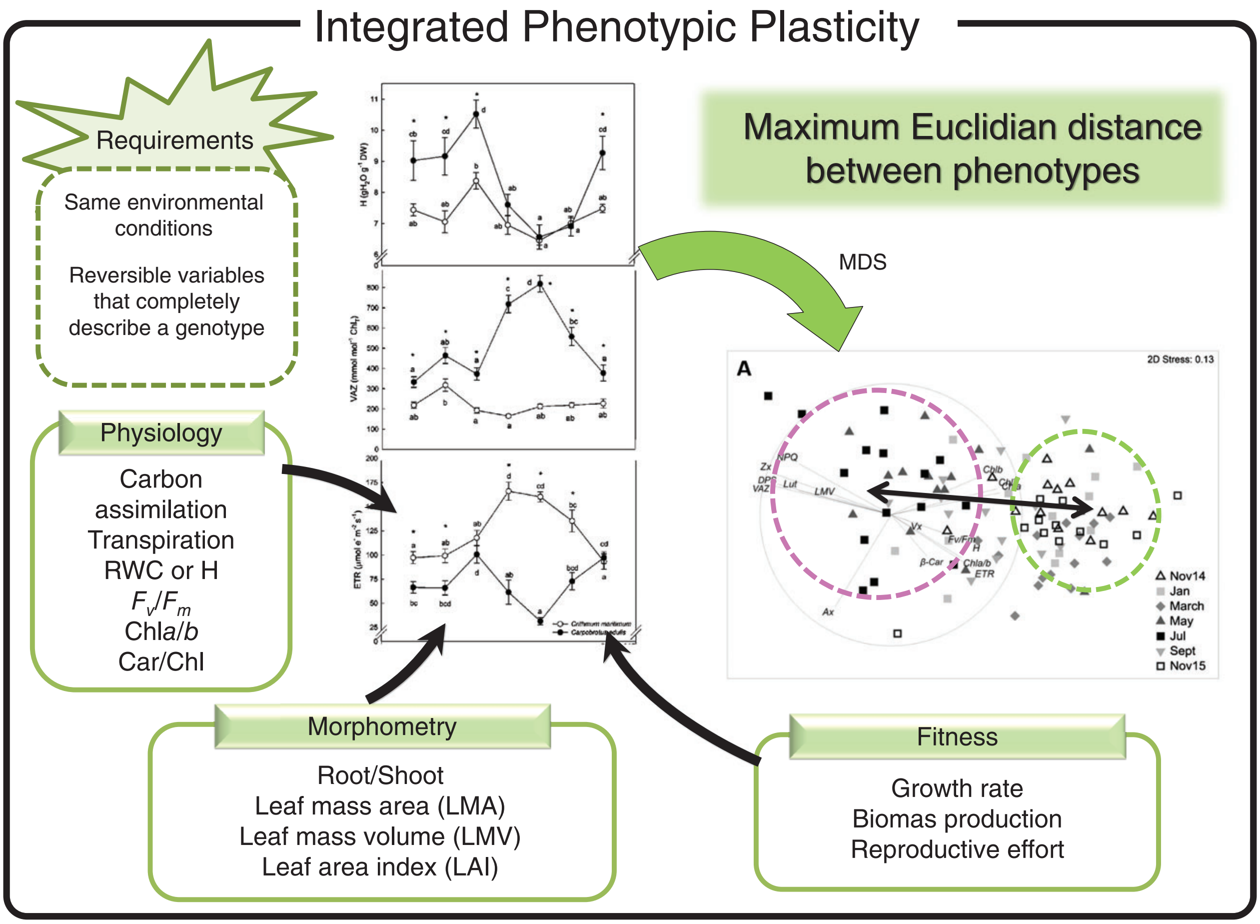

Fig. 7. Description of the integrated phenotypic plasticity index (PP_{int}), a proposed new approach to understanding phenotypic plasticity using some simple variables to ensure a complete phenotype description. RWC, relative water content; H, hydration; F_v/F_m, maximum quantum efficiency of photosystem II; Chl*a*/*b*, chlorophyll *a*/*b* ratio; Car/Chl, carotenoids/chlorophyll ratio; MDS, multidimensional scaling. Full MDS analyses are illustrated in Appendix S6.

(called jack-and-master) has been proposed for invasive plants (Richards et al. 2006). In invasive species, evolutionary changes to locally adapt can occur rapidly (García-Ramos et al. 2002). Despite being outside their biogeographic distribution range, a recent study postulates that invasive plant species are locally adapted just as frequently and at least as strongly as native plant species (Oduor et al. 2016). By contrast, some studies have found no local physiological adaptation, but high fitness under different environmental conditions in invasive species (Parker et al. 2003, Pahl et al. 2013). The question of whether or not higher plasticity makes an invader more aggressive or whether local adaptation is the crux of the matter remains open for debate (Liao et al. 2016). Recent studies on *Carpobrotus edulis* have provided evidence of rapid adaptive evolution (Roiloa et al. 2016), a key component for local adaptation (Colautti and Barrett 2013). Our study showed that *C. edulis* is perfectly adapted to Mediterranean conditions, especially to the Mediterranean summer, when this species takes advantage of xanthophylls to dissipate energy and photoprotect themselves. Compared to the native species, the invasive species presented a different but successful photoprotective strategy to cope with the annual variations in the environmental conditions in Cap de Creus. The native species seemed to suffer oxidative stress during the winter, while no signs of stress were observed in *C. edulis* during the winter. Thus, a shift in the climatic scenario, with a warmer winter season, could benefit this native species by ameliorating its winter stress, since it also maintains higher photosynthetic efficiency under the higher light levels in summer. If the results obtained are taken into account and an in situ approach used, it could be said that *C. edulis* showed higher plasticity in the parameters analyzed in response to the same climatic variations compared to *C. maritimum*, because the amplitude of its response was greater. However, this amplitude could also be categorized as susceptibility; *C. maritimum* maintained more constant values in the parameters analyzed over the course of a year. The native seemed to use a saving strategy by attempting to adjust to environmental changes with minimal variation in its physiological constraints, and only morphologic adaptation. *C. edulis* followed a reverse strategy; it seemed to use an all-in strategy whereby it responded to the annual variation though a plethora of photoprotection mechanisms. This strategy could be useful under a scenario of climatic unpredictability. The physiological costs of both strategies for the species and the growth repercussions require further investigation. Whether or not climate change will benefit the invader over the native species will depend on the magnitude and direction of the changes, but higher plasticity has been identified as a key factor in determining the suitability of species under this new scenario (Matesanz et al. 2010, Nicotra et al. 2010).

In conclusion, this work describes the different photoprotective strategies of *Carpobrotus edulis* and *Crithmum maritimum* in response to the annual variability of the environmental conditions on the Mediterranean coast. It also reveals that an invasive species, with considerably shorter local adaptation time, is perfectly adapted to the location studied, despite the fact that it uses a completely different photoprotective strategy to the native species. The reproductive and vegetative ramets of *C. edulis* also presented different investments in photoprotection, which led to functional heterogeneity in the same individual. The data obtained on photoprotective mechanisms made it possible to develop a new phenotypic plasticity index. This approach provides fresh insight into the invasion process through the use of ecophysiological markers that will be essential for further studies on the fitness of these species.

Author contributions

The work presented here was carried out in collaboration with all of the authors. E.F., S.M.B. and M.P.M. co-designed the experiments. S.M.B. obtained funding and contributed materials and reagents. E.F. and M.P.M. carried out the field sampling and laboratory experiments. E.F. analyzed the data. E.F., S.M.B. and M.P.M. discussed the analyses, interpreted the results and wrote the paper. All authors have contributed to, read and approved the manuscript.

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References

Adams WW, Zarter CR, Ebbert V, Demmig-Adams B (2004) Photoprotective strategies of overwintering evergreens. Bioscience 54: 41–49

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55: 373–399

Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds) The Genetics of Colonizing Species. Academic Press, New York, NY 147–172

Baraldi R, Canaccini F, Cortes S, Magnani F, Rapparini F, Zamboni A, Raddi S (2008) Role of xanthophyll cycle-mediated photoprotection in *Arbutus unedo* plants exposed to water stress during the Mediterranean summer. Photosynthetica 46: 378–386

Ben Hamed K, Castagna A, Salem E, Ranieri A, Abdelly C (2007) Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses. Plant Growth Regul 53: 185–194

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol 83: 887–889

Buck AL (1981) New equations for computing vapor pressure and enhancement factor. J Appl Meteorol 20: 1527–1532

Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers Distrib 15: 22–40

Ciccarelli D, Picciarelli P, Bedini G, Sorce C (2015) Mediterranean sea cliff plants: morphological and physiological responses to environmental conditions. J Plant Ecol 9: 153–164

Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342: 364–366

Conser C, Connor EF (2009) Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. Biol Invasions 11: 349–358

Croce R, Van Amerongen H (2011) Light-harvesting and structural organization of Photosystem II: From individual complexes to thylakoid membrane. J Photochem Photobiol B 104: 142–153

D'Antonio CM, Mahall BE (1991) Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. Am J Bot 78: 885–894

Dall'Osto L, Lico C, Alric J, Giuliano G, Havaux M, Bassi R (2006) Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection *in vivo* under strong light. BMC Plant Biol 6: 32

Davies PJ, Gan S (2012) Towards an integrated view of monocarpic plant senescence. Russ J Plant Physiol 59: 467–478

Demmig-Adams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol 43: 599–626

Demmig-Adams B, Adams WW (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci 1: 21–26

de la Peña E, de Clercq N, Bonte D, Roiloa S, Rodríguez-Echeverría S, Freitas H (2010) Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*. Biol Invasions 12: 3637–3648

Earnshaw MJ, Carver KA, Charlton WA (1987) Leaf anatomy, water relations and crassulacean acid metabolism in the chlorenchyma and colourless internal water-storage tissue of *Carpobrotus edulis* and *Senecio mandraliscae*. Planta 170: 421–432

Esteban R, Olano JM, Castresana J, Fernández-Marín B, Hernández A, Becerril JM, García-Plazaola JI (2009) Distribution and evolutionary trends of photoprotective isoprenoids (xanthophylls and tocopherols) within the plant kingdom. Physiol Plant 135: 379–389

Evans JR (1988) Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. Funct Plant Biol 15: 93–106

Fenollosa E, Roach DA, Munné-Bosch S (2016) Death and plasticity in clones influence invasion success. Trends Plant Sci 21: 551–553

Funk JL (2008) Differences in plasticity between invasive and native plants from a low resource environment. J Ecol 96: 1162–1173

García-Ramos G, Rodríguez D (2002) Evolutionary speed of species invasions. Evolution 56: 661–668

Grassein F, Till-Bottraud I, Lavorel S (2010) Plant resource-use strategies: the importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. Ann Bot 106: 637–645

Havaux M (2014) Carotenoid oxidation products as stress signals in plants. Plant J 79: 597–606

Hulme PE (2007) Phenotypic plasticity and plant invasions: is it all Jack? Funct Ecol 22: 3–7

Jahns P, Holzwarth AR (2012) The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim Biophys Acta 1817: 182–193

Jallali I, Megdiche W, M'Hamdi B, Oueslati S, Smaoui A, Abdelly C, Ksouri R (2012) Changes in phenolic composition and antioxidant activities of the edible halophyte *Crithmum maritimum* L. with physiological stage and extraction method. Acta Physiol Plant 34: 1451–1459

Jallali I, Zaouali Y, Missaoui I, Smeoui A, Abdelly C, Ksouri R (2014) Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoïdes* L. Food Chem 145: 1031–1038

Juvany M, Müller M, Munné-Bosch S (2013) Photo-oxidative stress in emerging and senescing leaves: A mirror image. J Exp Bot 64: 3087–3098

Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, Brestic M, Bussotti F, Calatayud A, Dąbrowski P, Elsheery NI, Ferroni L, Guidi L, Hogewoning SW, Jajoo A, Misra AN, Nebauer SG, Pancaldi S, Penella C, Poli D, Pollastrini M, Romanowska-Duda ZB, Rutkowska B, Serôdio J, Suresh K, Szulc W, Tambussi E, Yanniccari M, Zivcak M (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynth Res 122: 121–158

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7: 1225–1241

Liao H, D'Antonio CM, Chen B, Huang Q, Peng S (2016) How much do phenotypic plasticity and local genetic variation contribute to phenotypic divergences along environmental gradients in widespread invasive plants? A meta-analysis. Oikos 125: 1–13

Matesanz S, Gianoli E, Valladares F (2010) Global change and the evolution of phenotypic plasticity in plants. Ann NY Acad Sci 1206: 35–55

Matos FS, Wolfgramm R, Gonçalves FV, Cavatte PC, Ventrella MC, DaMatta FM (2009) Phenotypic plasticity in response to light in the coffe tree. Environ Exp Bot 67: 421–427

Meinzer FC (2003) Functional convergence in plant responses to the environment. Oecologia 134: 1–11

Meot-Duros L, Magné C (2009) Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. Plant Physiol Biochim 47: 37–41

Míguez F, Fernández-Marín B, Becerril JM, García-Plazaola JI (2015) Activation of photoprotective winter photoinhibition in plants from different environments: A literature compilation and meta-analysis. Physiol Plant 155: 414–423

Morales M, Pintó-Marijuan M, Munné-Bosch S (2016) Seasonal, sex- and plant size-related effects on photoinhibition and photoprotection in the dioecious mediterranean dwarf palm, *Chamaerops humilis*. Front Plant Sci 7: 1116

Morosinotto T, Bassi R (2012) Assembly of light harvesting pigment-protein complexes in photosynthetic eukaryotes. In: Eaton-Rye JJ, Tripathy BC, Sharkey TD (eds) Photosynthesis. Plastid Biology, Energy Conversion and Carbon Assimilation. Springer, Dordrecht, pp 113–126

Munné-Bosch S, Alegre L (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210: 925–931

Munné-Bosch S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. Funct Plant Biol 31: 203–216

Munné-Bosch S, Peñuelas J (2003) Photo- and antioxidative protection during summer leaf senescence in *Pistacia lentiscus* L. grown under Mediterranean field conditions. Ann Bot 92: 385–391

Munné-Bosch S, Peñuelas J (2004) Drought-induced oxidative stress in strawberry tree (*Arbutus unedo* L.) growing in Mediterranean field conditions. Plant Sci 166: 1105–1110

Munné-Bosch S, Jubany-Marí T, Alegre L (2001) Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. Plant Cell Environ 24: 1319–1327

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15: 684–692

Nisar N, Li L, Lu S, Khin NC, Pogson BJ (2015) Carotenoid metabolism in plants. Mol Plant 8: 68–82

Novoa A, González L (2014) Impacts of *Carpobrotus edulis* (L.) N.E.Br. on the germination, establishment and survival of native plants: a clue for assessing its competitive strength. PLoS Biol 9: e107557

Novoa A, González L, Moravcová L, Pyšek P (2013) Constraints to native plant species establishment in coastal dune communities invaded by *Carpobrotus edulis*: Implications for restoration. Biol Conserv 164: 1–9

Oduor AMO, Leimu R, van Kleunen M (2016) Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. J Ecol 104: 957–968

Oliveira G, Peñuelas J (2004) Effects of winter cold stress on photosynthesis and photochemical efficiency of PSII of the Mediterranean *Cistus albidus* L. and *Quercus ilex* L. Plant Ecol 175: 179–191

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – Calculation of qP and Fv′/Fm′ without measuring Fo′. Photosynth Res 54: 135–142

Pahl AT, Kollmann J, Mayer A, Haider S (2013) No evidence for local adaptation in an invasive alien plant: Field and greenhouse experiments tracing a colonization sequence. Ann Bot 112: 1921–1930

Parker IM, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conserv Biol 17: 59–72

Pastenes C, Pimentel P, Lillo J (2005) Leaf movements and photoinhibition in relation to water stress in field-grown beans. J Exp Bot 56: 425–433

Peperkorn R, Werner C, Wolfram B (2005) Phenotypic plasticity of an invasive acacia versus two native Mediterranean species. Funct Plant Biol 32: 933–944

Pintó-Marijuan M, Munné-Bosch S (2013) Ecophysiology of invasive plants: osmotic adjustment and antioxidants. Trends Plant Sci 18: 660–666

Pintó-Marijuan M, Munné-Bosch S (2014) Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: advantages and limitations. J Exp Bot 65: 3845–3857

Queiroz O, Brulfert J (1982) Photoperiod-controlled induction and enhancement of seasonal adaptation to drought. In: Ting I, Gibbs M (eds) Crassulacean acid metabolism. American Society of Plant Physiologists, Rockville, MD, pp 208–230

Ramel F, Birtic S, Cuiné S, Triantaphylidès C, Ravanat J-L, Havaux M (2012a) Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiol 158: 1267–1278

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidès C, Havaux M (2012b) Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc Natl Acad Sci USA 109: 5535–5540

Ramel F, Mialoundama AS, Havaux M (2013) Nonenzymic carotenoid oxidation and photooxidative stress signalling in plants. J Exp Bot 64: 799–780

Raven JA (2011) The cost of photoinhibition. Physiol Plant 142: 87–104

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecol Lett 9: 981–993

Roiloa SR, Rodríguez-Echeverría S, de la Peña E, Freitas H (2010) Physiological integration increases the survival and growth of the clonal invader *Carpobrotus edulis*. Biol Invasions 12: 1815–1823

Roiloa SR, Rodríguez-Echeverría S, Freitas H, Retuerto R (2013) Developmentally-programmed division of labour in the clonal invader *Carpobrotus edulis*. Biol Invasions 15: 1895–1905

Roiloa SR, Retuerto R, Campoy JG, Novoa A, Barreiro R (2016) Division of labor brings greater benefits to clones of *Carpobrotus edulis* in the non-native range: evidence for rapid adaptive evolution. Front Plant Sci 7: 1–13

Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. Trends Ecol Evol 28: 58–66

Takahashi S, Badger MR (2011) Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci 16: 53–60

Thayer SS, Björkman O (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynth Res 23: 331–343

Thimann KV, Satler S (1979) Relation between senescence and stomatal opening: Senescence in darkness. Proc Natl Acad Sci USA 76: 2770–2773

Thomas H (2013) Senescence, ageing and death of the whole plant. New Phytol 197: 696–711

Triantaphylidès C, Havaux M (2009) Singlet oxygen in plants: production, detoxification and signaling. Trends Plant Sci 14: 219–228

Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94: 1103–1116

Vilà M, Tessier M, Gimeno I, Moragues E, Traveset A, de la Bandera MC, Suehs CM, Médail F, Affre L, Galanidis A, Dalias P, Petsikos B, Carta L, Manca M, Brundu G (2004) Impacts of plant invasion on species diversity in Mediterranean islands. In: Arianoutsou P (ed) Proceedings 10th MEDECOS Conference. Millpress, Rotterdam, pp 1–7

Wingler A, Marès M, Pourtau N (2004) Spatial patterns and metabolic regulation of photosynthetic parameters during leaf senescence. New Phytol 161: 781–789

Winter K (1973) Discovery of NaCl induced crassulacean acid metabolism in a second member of the Aizoaceae family: *Carpobrotus edulis*. Planta 115: 187–188

Winter K, Holtum JAM (2014) Facultative crassulacean acid metabolism (CAM) plants: Powerful tools for unravelling the functional elements of CAM photosynthesis. J Exp Bot 65: 3425–3441

Yamamoto HY, Bassi R (1996) Carotenoids: localization and function. In: Donald OR, Yocum CF (eds) Oxygenic Photosynthesis: The Light Reactions. Kluwer Academic Publishers, Dordrecht, pp 539–563

Zimmermann P, Zentgraf U (2005) The correlation between oxidative stress and leaf senescence during plant development. Cell Mol Biol Lett 10: 515–534

Zunzunegui M, Barradas MCD, Ain-Lhout F, Alvarez-Cansino L, Esquivias MP, Novo FG (2011) Seasonal physiological plasticity and recovery capacity after summer stress in Mediterranean scrub communities. Plant Ecol 212: 127–142

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Location of the three zones studied (Z1–3) in Far de s'Arenella (NE Spain).

Appendix S2. ANOVA *P* values results of the three factors analyzed and the interaction between the fixed factors for the presented variables.

Appendix S3. Climatic conditions at the station closest to the study site from November 2014 to November 2015.

Appendix S4. Pearson correlation matrix for *Carpobrotus edulis*.

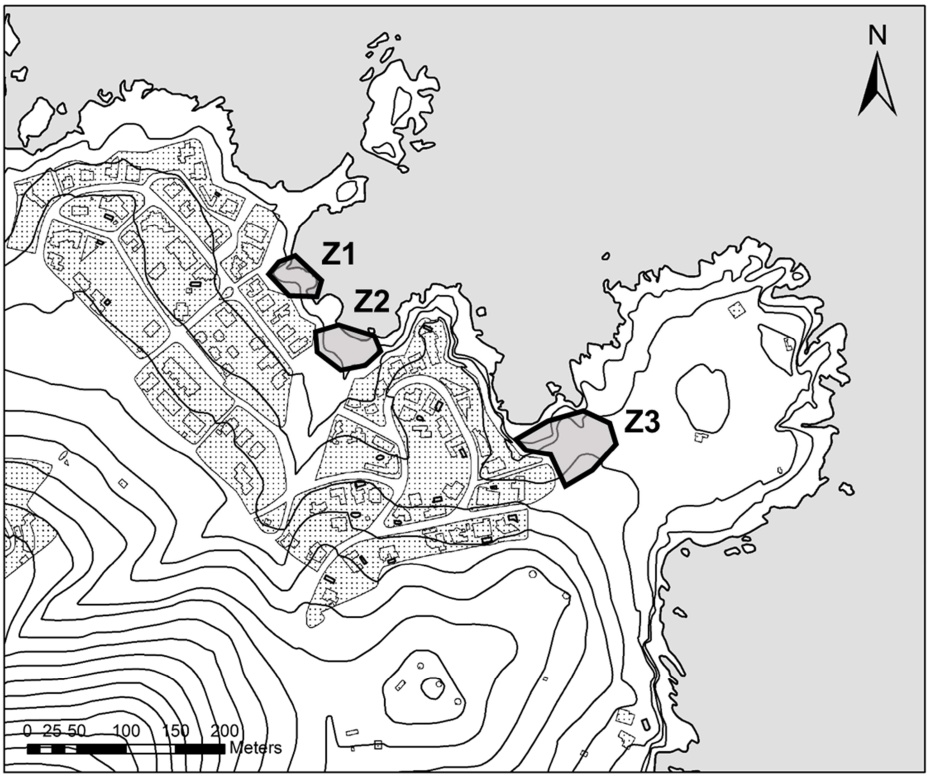
Appendix S5. Pearson correlation matrix for *Crithmum maritimum*.

Appendix S6. MDS plots for *Carpobrotus edulis* and Crithmum *maritimum*.

Appendix S7. PERMANOVA *P* values for comparison of each month.

Edited by A. Krieger-Liszkay

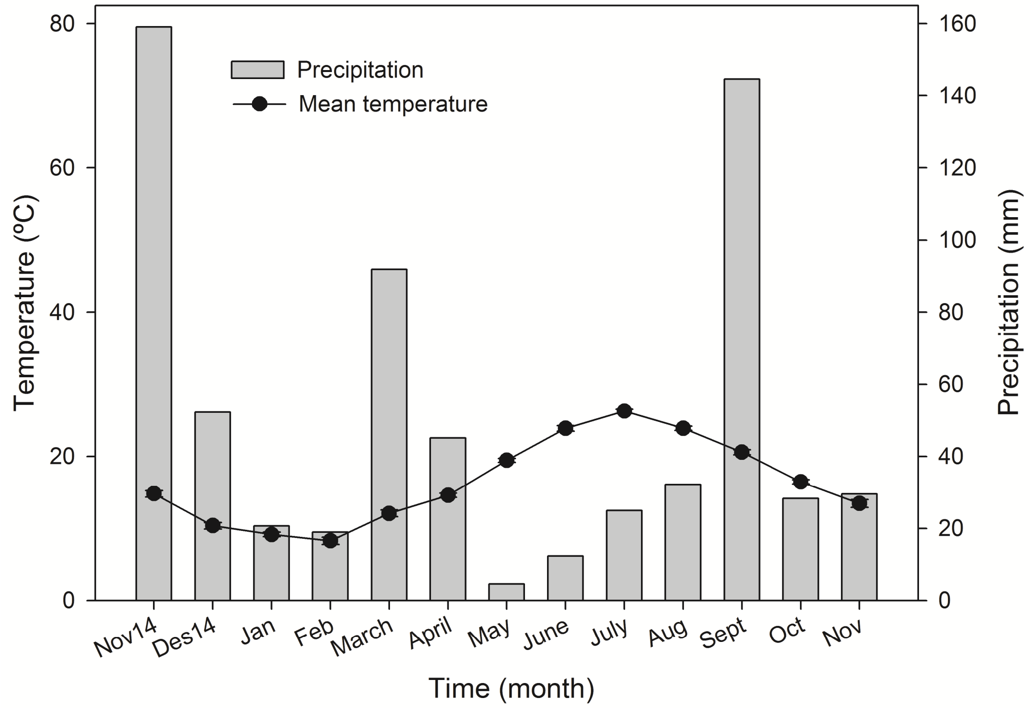
SUPPLEMENTARY INFORMATION



Appendix S1. Localization of the three considered zones (Z1-3) in Far de s’Arenella (NE Spain) (42°21'02.4"N 3°11'11.9"E). The distance between isopleths is 5m. Map bases are taken from *Institut Cartogràfic de Catalunya* (ICC).

Appendix S2. ANOVA *P*-value results of the three analyzed factors and the interaction between the fixed factors for the presented variables. In bold the significant effects (*P*-value< 0.05).

	Species	Month	Zone	Sp*Month
H ($gH_2O \cdot gDW^{-1}$)	0.155	**<0.001**	0.350	**0.015**
LMV ($gDW \cdot L^{-1}$)	0.212	**<0.001**	0.380	**<0.001**
ETR ($\mu mol\ e^{-} \cdot m^{-2} \cdot s^{-1}$)	**0.021**	0.308	0.552	**0.001**
F_v/F_m	**0.017**	**0.021**	0.125	**0.026**
NPQ	**0.030**	**0.027**	0.437	**0.001**
ChlT ($mmol \cdot m^{-2}$)	**0.018**	0.159	0.850	0.199
Chl*a/b*	0.838	0.131	0.294	**<0.001**
VAZ ($mmol/molChl_T^{-1}$)	**0.010**	**0.001**	0.763	**<0.001**
DPS	**0.010**	**0.038**	0.455	**<0.001**
Lut ($mmol/molChl_T^{-1}$)	**0.056**	**0.004**	0.317	**0.104**
β-Car ($mmol/molChl_T^{-1}$)	**0.039**	0.546	0.473	**<0.001**



Appendix S3: Climatic conditions (mean temperature and monthly precipitation) recorded in the nearest station from the study site, in Roses (42°16'01.8"N 3°13'26.1"E) from November 2014 to November 2015. This data was provided by Climatic Services *of Servei Meteorològic de Catalunya*.

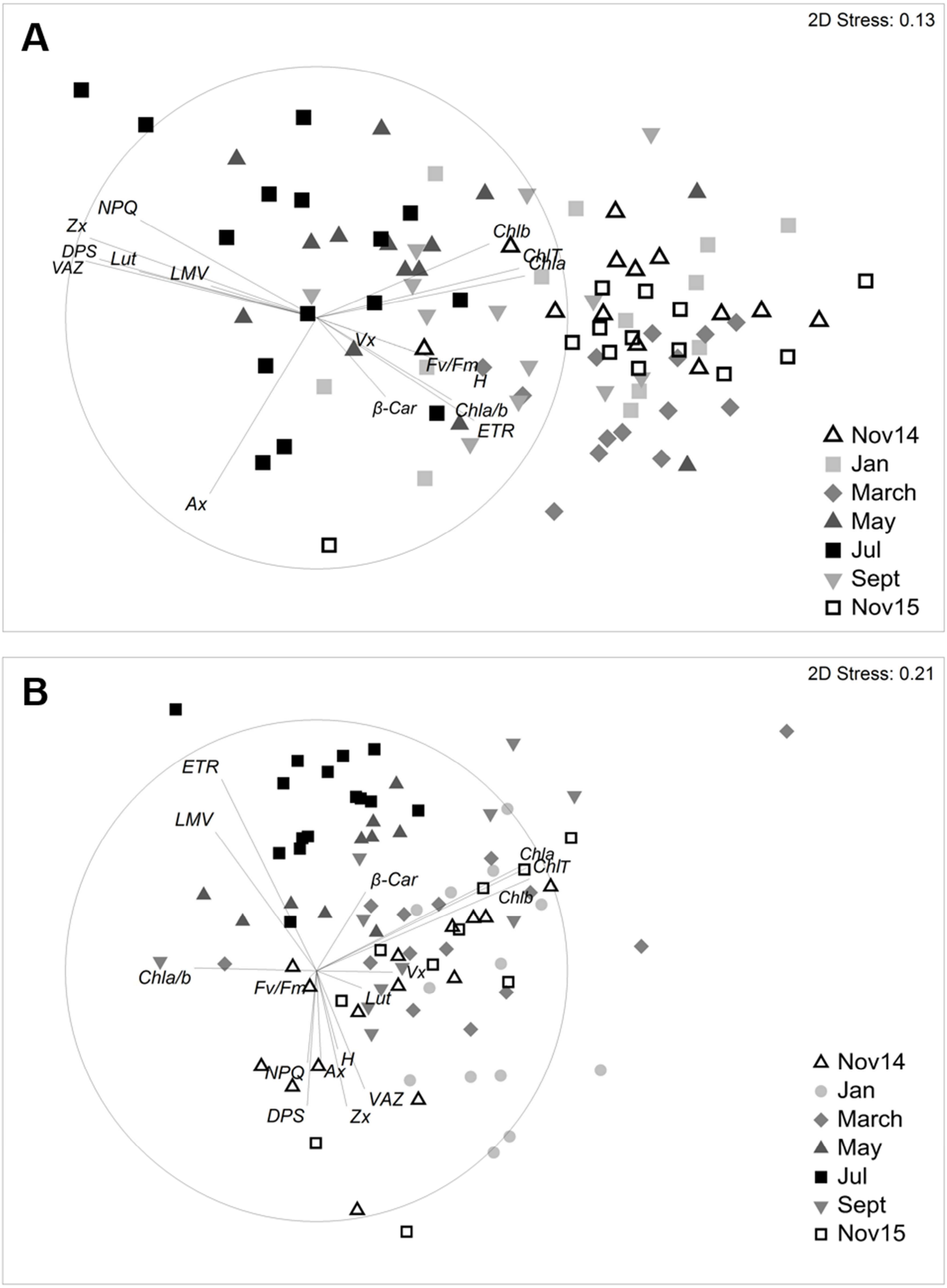
Appendix S4. Pearson correlation matrix for *C. edulis*, with the correlation coefficient and *P*-value in brackets. Significant correlations (in grey), are considered applying Bonferroni's *P*-value adjustment (*P*-value< 0.0045).

	LMV ($g \cdot L^{-1}$)	**ETR** ($\mu mol\ e^{-} \cdot m^{-2} \cdot s^{-1}$)	**F_v/F_m**	**NPQ**	**Chl_T** ($mmols \cdot m^{-2}$)	**Chl*a*/*b***	**VAZ** ($mmols \cdot mol\ Chl_T^{-1}$)	**DPS** ($Z+0.5 \cdot A/VAZ$)	**Lut** ($mmols \cdot mol\ Chl_T^{-1}$)	**β-Car** ($mmols \cdot mol\ Chl_T^{-1}$)
H ($gH_2O \cdot gDW^{-1}$)	-0.693 (<0.001)	0.337 (<0.001)	0.387 (<0.001)	-0.479 (<0.001)	0.368 (<0.001)	0.575 (<0.001)	-0.563 (<0.001)	-0.561 (<0.001)	-0.451 (<0.001)	0.318 (0.001)
LMV ($g \cdot L^{-1}$)		-0.334 (<0.001)	-0.391 (<0.001)	0.254 (0.005)	-0.279 (0.002)	-0.560 (<0.001)	0.348 (<0.001)	0.254 (0.006)	0.387 (<0.001)	-0.423 (<0.001)
ETR ($\mu mol\ e^{-} \cdot m^{-2} \cdot s^{-1}$)			0.360 (<0.001)	-0.565 (<0.001)	0.354 (<0.001)	0.500 (<0.001)	-0.582 (<0.001)	-0.544 (<0.001)	-0.458 (<0.001)	0.351 (<0.001)
F_v/F_m				-0.295 (0.001)	0.336 (<0.001)	0.342 (<0.001)	-0.395 (<0.001)	-0.352 (<0.001)	-0.456 (<0.001)	0.282 (0.002)
NPQ					-0.446 (<0.001)	-0.426 (<0.001)	0.688 (<0.001)	0.608 (<0.001)	0.441 (<0.001)	-0.363 (<0.001)
Chl_T ($mmols \cdot m^{-2}$)						0.161 (0.050)	-0.583 (<0.001)	-0.642 (<0.001)	-0.575 (<0.001)	0.075 (-0.2267)
Chl*a*/*b*							-0.561 (<0.001)	-0.441 (<0.001)	-0.465 (<0.001)	0.533 (<0.001)
VAZ ($mmols \cdot mol\ Chl_T^{-1}$)								0.873 (<0.001)	0.672 (<0.001)	-0.332 (<0.001)
DPS ($Z+0.5 \cdot A/VAZ$)									0.560 (<0.001)	-0.195 (0.025)
Lut ($mmols \cdot mol\ Chl_T^{-1}$)										-0.130 (0.096)

Appendix S5. Pearson correlation matrix for *C. maritimum*, with the correlation coefficient and P-value in brackets. Significant correlations (in grey), are considered applying Bonferroni's P-values adjustment (P-value < 0.0045).

	LMV ($g \cdot L^{-1}$)	**ETR** ($\mu mol\ e^{-} \cdot m^{-2} \cdot s^{-1}$)	**F_v/F_m**	**NPQ**	**Chl_T** ($mmols \cdot m^{-2}$)	**Chl*a*/*b***	**VAZ** ($mmols \cdot mol\ Chl_T^{-1}$)	**DPS** ($Z+0.5 \cdot A/VAZ$)	**Lut** ($mmols \cdot mol\ Chl_T^{-1}$)	**β-Car** ($mmols \cdot mol\ Chl_T^{-1}$)
H ($gH_2O \cdot gDW^{-1}$)	-0.561 (<0.001)	-0.245 (0.007)	0.029 (0.386)	0.253 (0.006)	0.017 (0.434)	-0.136 (0.084)	-0.068 (0.247)	0.040 (0.346)	-0.176 (0.038)	-0.323 (<0.001)
LMV ($g \cdot L^{-1}$)		0.420 (<0.001)	-0.006 (0.477)	-0.084 (0.207)	-0.178 (0.036)	0.252 (0.005)	-0.005 (0.482)	-0.160 (0.054)	0.121 (0.111)	0.377 (<0.001)
ETR ($\mu mol\ e^{-} \cdot m^{-2} \cdot s^{-1}$)			-0.110 (0.137)	-0.414 (<0.001)	0.075 (0.228)	0.139 (0.081)	-0.278 (0.002)	-0.216 (0.016)	0.037 (0.357)	0.071 (0.237)
F_v/F_m				0.122 (0.115)	0.052 (0.299)	0.060 (0.273)	-0.056 (0.286)	0.081 (0.210)	-0.073 (0.231)	-0.004 (0.483)
NPQ					-0.174 (0.043)	-0.255 (0.006)	-0.043 (0.339)	-0.058 (0.289)	-0.016 (-0.440)	-0.228 (0.012)
Chl_T ($mmols \cdot m^{-2}$)						-0.377 (<0.001)	-0.111 (0.132)	-0.214 (0.015)	-0.022 (0.412)	0.121 (0.110)
Chl*a*/*b*							-0.073 (0.232)	0.188 (0.028)	-0.220 (0.012)	0.274 (0.002)
VAZ ($mmols \cdot mol\ Chl_T^{-1}$)								0.736 (<0.001)	0.623 (<0.001)	0.298 (0.001)
DPS ($Z+0.5 \cdot A/VAZ$)									0.249 (0.006)	0.151 (0.064)
Lut ($mmols \cdot mol\ Chl_T^{-1}$)										0.429 (<0.001)

Appendix S6. Multidimensional scaling analysis (MDS) for *Carpobrotus edulis* (A) and *Crithmum maritimum* (B). Variables are shown as vectors, whose length and direction reflect their influence on data variability.



Appendix S7. PERMANOVA P-values derived from the euclidian distance matrix between samples for *Carpobrotus edulis* and *Crithmum maritimum* for all the compared months. The number of permutations was 1000.

Groups	*C. edulis*	*C. maritimum*
1_Nov14, 2_Jan	0.124	<0.001
1_Nov14, 3_March	<0.001	0.032
1_Nov14, 4_May	<0.001	<0.001
1_Nov14, 5_Jul	<0.001	<0.001
1_Nov14, 6_Sept	<0.001	<0.001
1_Nov14, 7_Nov15	0.221	0.765
2_Jan, 3_March	0.039	<0.001
2_Jan, 4_May	0.005	<0.001
2_Jan, 5_Jul	<0.001	<0.001
2_Jan, 6_Sept	0.037	0.009
2_Jan, 7_Nov15	0.148	0.019
3_March, 4_May	<0.001	<0.001
3_March, 5_Jul	<0.001	<0.001
3_March, 6_Sept	<0.001	<0.001
3_March, 7_Nov15	0.096	0.224
4_May, 5_Jul	<0.001	<0.001
4_May, 6_Sept	0.004	<0.001
4_May, 7_Nov15	<0.001	<0.001
5_Jul, 6_Sept	<0.001	<0.001
5_Jul, 7_Nov15	<0.001	<0.001
6_Sept, 7_Nov15	0.004	0.026

Capítol 2. *Re-greening* per efectes ambientals en ramets senescents d'una planta invasora

Chapter 2. Environmental driven re-greening of senescing ramets in an invasive plant, *Carpobrotus edulis*: Occurrence, hormonal regulation and biological significance.

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Environmental driven re-greening of senescing ramets in an invasive plant: occurrence, hormonal regulation and biological significance

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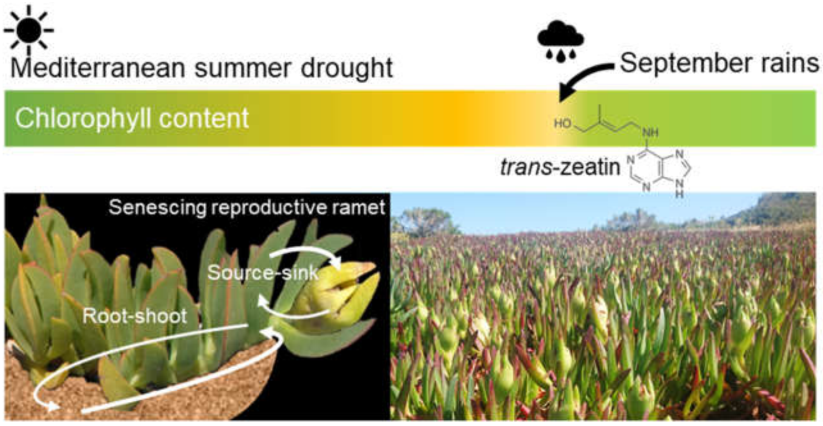
Keywords: Aizoaceae, chlorophyll loss, clonal, phytohormones, plasticity, reproductive effort, zeatin.

Abstract

Plants undergo leaf senescence after fruit formation through a delicate hormonal regulation balancing leaf senescence with nutrient remobilization to the seeds. However, the reversibility of the leaf senescence process (re-greening) and its regulatory mechanisms, either internal (e.g. by hormones) or external (e.g. by the environment) have been poorly studied in perennial plants, most particularly under natural field conditions. In this study, a highly plastic invasive species, *Carpobrotus edulis* (L.) N. E. Br. was used to study the occurrence, regulatory mechanisms and possible biological significance of ramet senescence in plants growing in their natural habitat. We examined the occurrence of re-greening under field conditions, its relationship with the prevailing environmental conditions and regulation by cytokinins, and its possible biological significance by testing the influence of the reproductive load in plants growing under Mediterranean field conditions. Results showed that re-greening of senescing monocarpic reproductive ramets is possible in plants growing under natural conditions when rainfall allows drought recovery during late summer. The accumulation of *trans*-zeatin (an active cytokinin) after rainfall allowed re-greening in senescing leaves from reproductive ramets. In conclusion, re-greening in senescing leaves occurs in the invasive plant *C. edulis* in a process that appears to be mediated not only by the environmental conditions but also by cytokinins. This mechanism may allow resuming seed filling in periods of unpredictable water shortage in the frame of climate change.



1. Introduction

In spite of the fact of the exceptional biodiversity found in the Mediterranean Basin, plant survival is constrained by the unusual temporal dissociation along the year between growth optimal temperature and water availability that is coped through highly diverse photoprotective strategies (Fernandez-Marín et al. 2018). A drought period, intensive radiation and moderately to extremely high temperatures characterize Mediterranean summers (Köppen 1923). Climatic models suggest that drought episodes will become more frequent because of the long-term effects of global warming threatening actual biodiversity (Christensen et al. 2007). Plants ability to deal with photooxidative stress derived from stochastic water availability regimes and avoid or further repair photooxidative damage will define its stress tolerance and plant survival capacity to harsh periods (McDowell et al. 2008). Low internal CO_2 concentrations as a consequence of stomatal closure under drought stress leads to an accumulation of $NADPH^+$ at the electron transport chain collapsing the photosynthetic apparatus by an energy excess that leads to photo-oxidative stress (Takahashi and Badger 2011). If this is not counterbalanced by photoprotective strategies including a boost in antioxidant defenses, it rapidly leads to photo-oxidative damage and unprogrammed cell death occurs, so that drought-induced leaf senescence cannot provide an adaptative role in terms of nutrient reallocation to productive leaves (Munné-Bosch and Alegre 2004). Chlorophyll loss is a process associated with both stress and senescence processes and among the mechanisms to prevent photo-oxidative stress, it plays an essential role (Zimmermann and Zentgraf 2005). As summarized in Munné-Bosch and Alegre (2004), a reduction of leaf chlorophyll content plays a triple role during the first stages of drought-induced leaf senescence: (i) reduces potentially harmful effects of singlet oxygen formation in thylakoids, (ii) reduces the amount of light absorbed by leaves, and (iii) contributes to increase the amounts of carotenoids and tocopherols per unit of chlorophyll, increasing the photoprotective and antioxidant capacity relative to the absorbed light (Kyparissis et al. 1995, Krieger-Liszkay 2005, Takahashi and Badger 2011).

Despite the well-known plant drought responses, variations in drought tolerance may be found within an individual because of extrinsic (e.g. environmental gradients) and intrinsic factors (e.g. developmental differences, epigenetic changes) (Herrera 2017). For instance, the recent study developed by Cardoso et al. (2020) described high interleaf variability in xylem resistance to embolism in *Persea americana* that shows a strong negative correlation with leaf necrosis, driving temporal and spatial patterns of drought-induced leaf senescence within an individual. Among intrinsic factors determining within-individual variability, the costs of reproduction may condition adjacent plant organs stress responses (Obeso 2002). Increased reproductive effort is proposed to promote a physiological deterioration of leaves due to limited resource availability (Wingler et al. 2006). Those reproduction costs are especially observable in monocarpic species as once whole plant developmental senescence has been initiated, it leads to a massive remobilization of phloem-mobile nutrients from the senescing plant parts to developing sinks, such as seeds (Noodén 2004, Thomas 2013). Cytokinins constitute main internal regulators of the source-sink dynamics during nutrient remobilization and are the most potent general antagonists of senescence and therefore play an important role controlling the senescence timing (Zwack and Rashotte 2013). Developmental leaf senescence in monocarpic plants may be altered also by external factors after certain environmental cues such as drought, that accelerates the time-course of monocarpic senescence in several species so that plant reproduction may be achieved even under stressful conditions (Yang and Zhan 2006). In spite of the adaptative value of this external regulation, seed composition can be drastically impacted at various cellular levels due to drought and heat stresses (Sehgal et al. 2018), and eventually lead to seed viability loss (Su et al. 2013). The plasticity of intrinsic and extrinsic regulatory mechanisms influencing leaf senescence has been poorly explored in non-model species under natural conditions and may have an important biological significance resuming seed filling.

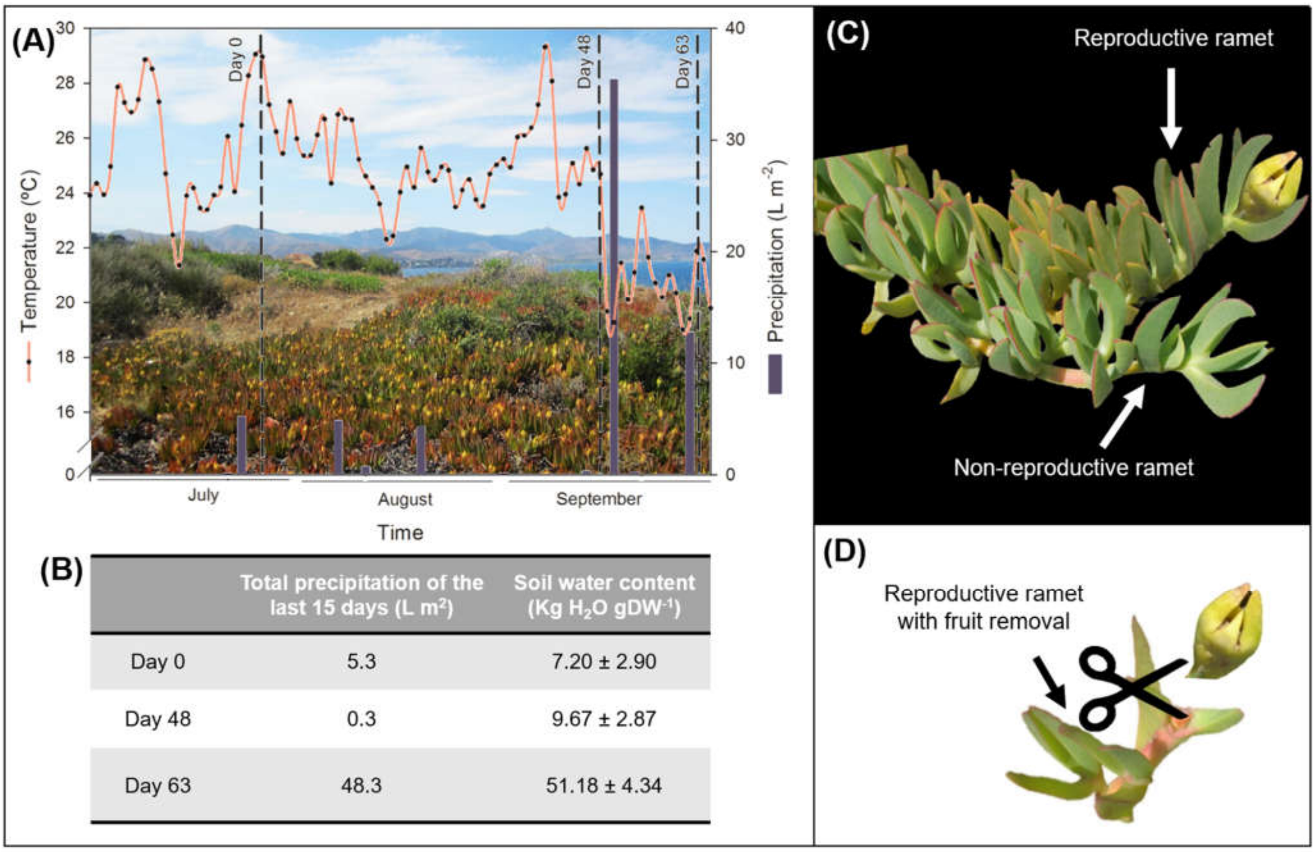
Both drought-induced leaf senescence and developmental senescence may potentially occur during Mediterranean summers in the monocarpic clonal species *Carpobrotus edulis* (L.) N. E. Br. (Aizoaceae). *C. edulis* is a succulent mat-forming clonal plant native from South Africa that has become invasive in different coastal Mediterranean regions around the world (Vilà et al. 2008). Besides a vigorous clonal growth (Roiloa et al. 2019) that allows this species to sustain growth in hostile habitats making them habitable after successive death cycles (Fenollosa et al. 2016), *C. edulis* has also a high sexual potential showing high flower and seed production with high seed longevity, that strongly contributes to this species invasive success (Chenot *et al*. 2014). A *C. edulis* genet (clonally identical individual) is composed of two ramet types (potentially isolable units): reproductive (with apical fruit) and non-reproductive ramets (Fenollosa et al. 2016). The senescence of the reproductive ramets takes place between July and December, ending with ramet death and completely dehydrated fruits that fall and contribute to the seed soil bank (Fenollosa et al. 2017; Chenot *et al*. 2014). Therefore, the initiation of the fruit maturation phase concurs with this highly stressful period at the Mediterranean Basin (Mitrakos 1980).

In this study, we aimed to evaluate (1) the role of reproduction on the leaves photoprotective potential in response to drought stress under natural conditions, and (2) the existence of plastic responses regarding developmental and drought-induced senescence and its regulatory mechanisms via cytokinins. For those purposes we monitored reproductive and non-reproductive ramets of *C. edulis* under natural Mediterranean conditions, before and after a stress period. Fruit was removed in some *C. edulis* separated patches to evaluate their role on stress responses. We hypothesized that (1) leaves from reproductive ramets may show increased photoprotection to face and control the developmental senescence, (2) drought stress in reproductive ramets may accelerate senescence via cytokinin regulation, (3) fruit removal may delay the senescence process, and (4) reproductive load may compromise the ramet recovery after stress.

2. Material and Methods

2.1. Reproductive versus non-reproductive ramets

Twenty separated patches of *Carpobrotus edulis* (L.) N. E. Br. were selected within the area of Far de s'Arenella (NE Spain) (42°21'01.2", N 3°11'12.4" E). Two types of leaves were taken within each patch: leaves from ramets with fruits (reproductive senescing ramets) and leaves from proximal ramets (within a 20cm diameter) without fruits (non-reproductive ramets) (**Fig. 1C**). *C. edulis*



	Total precipitation of the last 15 days (L m^2)	Soil water content (Kg H_2O gDW^{-1})
Day 0	5.3	7.20 ± 2.90
Day 48	0.3	9.67 ± 2.87
Day 63	48.3	51.18 ± 4.34

Figure 1. Experimental design: Mediterranean drought and *C. edulis* ramet types. (A) Daily mean temperature and precipitations from July to October 2016. **(B)** Cumulative precipitation of the last 15 days and soil water content (Mean ± SE, N=6) at each sampling day. **(C)** Reproductive and non-reproductive ramets in *C. edulis.* **(D)** Fruit removal treatment in *C. edulis* reproductive ramets.

patches were sampled three times during the 2016 Mediterranean summer: in July (Day 0), after almost two months with little rains (Day 48) and after September rains (Day 63) (**Fig. 1A**). Day 0 was the first clear day after the unique monthly rains. Six soil samples randomly taken within the sampling area were used to quantify soil water content by the difference between ca. 30g of fresh weight soil and its dry weight (48 h at 60ºC until constant weight). July was chosen after previous studies that found differentiation between reproductive and non-reproductive *C. edulis* leaves in that month, when the plant starts the remobilization process and the fruit starts shifting from green to yellow-red-brownish colors (Fenollosa et al. 2017). Samplings were performed at midday time on sunny and clear days. Three fully developed leaves with direct incident light at the last 10cm of the branch tip were chosen. Two leaves were frozen in liquid nitrogen and stored at -80ºC to measure photo- and antioxidant protection, lipid peroxidation and cytokinins contents, and one was used for leaf hydration (H) and the maximum quantum efficiency of photosystem II (F_v/F_m) measurement.

2.2. Fruit removal experiment

In addition to the twenty randomly selected patches to evaluate the difference between reproductive and vegetative ramets, twenty additional *C. edulis* patches were selected to evaluate the effects of fruit removal on the senescence time-course of the reproductive ramets. This treatment consisted on the detachment of all reproductive organs of reproductive ramets within a diameter of 2 m from a labelled reproductive ramet (**Fig. 1D**). Fruit removal was performed immediately after a first sampling at Day 0 to evaluate initial differences. After 48 days with little rains and after September rains fruit-removed reproductive ramets were sampled again. In the same way for the comparison of reproductive and non-reproductive ramets, at the three sampling days, leaf hydration and F_v/F_m were measured and two leaves from fruit-removed reproductive ramets were frozen in liquid nitrogen and stored at -80ºC to measure photo- and antioxidant protection, lipid peroxidation and cytokinins contents.

2.3. Reproductive effort estimation

At the first day, *C. edulis* patches were characterized by reproductive effort, estimated as weighted fruit density (Kg of fruits m^{-2}). Fruit number was counted using a 1 m^2 quadrant. Five fruits per *C. edulis* patch were taken to measure mean fruit fresh weight. To evaluate the existence of plant stress responses compromises with reproductive effort, correlation analysis was performed, including loss and recovery percentages for each measured parameter. Loss and recovery percentages were calculated with the percentual loss from 0 to 48 days and the percentual gain from 48 to 63 days respectively.

2.4. Leaf hydration and F_v/F_m

To measure hydration, leaf fresh weight (FW) and dry weight (DW) (over-drying the tissue at 60°C until constant weight) were measured and leaf hydration was determined as H = (FW-DW)/DW. Measurement of the maximum quantum efficiency of photosystem II (F_v/F_m) was performed after 1h of dark adaptation, with MiniPam (Walz, Effeltrich, Germany).

2.5. Photo- and antioxidant protection and lipid peroxidation

Leaf samples were ground in liquid nitrogen using a mix ball and extracted with cold methanol containing 0.01% butylated hydroxytoluene (BHT, w/v) using ultrasonication. After centrifuging at 14 000 *g* for 10 min at 4°C, the supernatant was collected, and the pellet re-extracted with the same solvent until it was colorless. Supernatants were pooled and filtered with 0.22 µm PTFE filters (Phenomenex, Torrance, USA). Using the fresh resultant plant extract, different techniques were used to quantify chlorophylls, carotenoids, alpha tocopherol, the accumulation of lipid hydroperoxides and cytokinin contents. Chlorophylls and total carotenoids were measured spectrophotometrically following Lichtenthaler and Wellburn (1983). Leaf area per fresh weight was measured at each leaf before grounding through image analysis performed with ImageJ Software (NIH, Bethesda, USA).

The lipophilic antioxidant alpha tocopherol was separated isocratically in a normal-phase high performance liquid chromatography (HPLC) and quantified with a fluorescent detector, as described by Amaral et al. (2005). Briefly, the equipment consists of an integrated Jasco (Tokyo, Japan) system, with a PU-2089 Plus pump, a AS-2055 Plus auto-sampler and a FP-1520 fluorescence detector. An Inertsil 100A (5µm, 30 × 250mm, GL Sciences Inc., Tokyo, Japan) normal phase column operating at room temperature was used as stationary phase and a 0.7 mL min^{-1} mixture of n-hexane and p-dioxane (95.5; 4.5 v/v) was used as a mobile phase. Quantification was based on the results obtained from the fluorescence signal and compared to that of a calibration curve made with an authentic standard (Sigma-Aldrich, Steinheim, Germany).

The extent of lipid peroxidation was estimated spectrophotometrically as the accumulation of lipid hydroperoxides following the modified ferrous oxidation-xylenol orange (FOX) assay as described in DeLong et al. (2002). Briefly, two aliquots of the fresh extract were incubated for 30 min in darkness, one marked as positive (+), consisting of a mixture of 100µL of sample and 100µL of cold methanol with 0.01% BHT, and the other marked as negative (-), consisting of a mixture of 100µL of sample and 100µL of 10 mM triphenylphosphine (TPP). After that, three pseudo replicates per sample of 50 µL of + and – samples were incubated in 96 multi-well plates for 45 min at room temperature with 150 µL of freshly prepared FOX reagent consisting of 25 mM sulfuric acid, 4 mM BHT, 250µM ammonium iron (II) sulfate hexahydrate and 100µM xylenol orange. Measurements were made at 560 nm and determination was performed using the absorbance difference between (+) - (-) samples discarding 800 nm absorbance, using a H_2O_2 calibration curve as a standard that was incubated with the samples.

2.6. *Determination of cytokinins content*

Using the freshly prepared methanolic plant extract, the cytokinins *trans*-zeatin (*t*-Z), *trans*-zeatin riboside (*t*-ZR), isopentenyl adenine (2iP) and isopentenyl adenosine (IPA) were separated using an elution gradient based on 0.05% glacial acetic acid (solvent A) and acetonitrile with 0.05% glacial acetic acid (solvent B) at a constant flow rate of 0.6 ml min^{-1} on a reverse-phase UHPLC/ESI-MS/MS system. The liquid chromatography system consisted of an Aquity UHPLC TM System (Waters, Milford, MA USA) using a C18 Kinetex column (50 × 2.1 mm, 1.7 µm; Phenomenex, Macclesfield, UK). Cytokinins were quantified using an API3000 triple quadruple mass spectrometer (PE Sciex, Concord, Ontario, Canada) by using multiple reaction monitoring (MRM) in turbo Ionspray source in positive ion mode as described by Müller and Munné-Bosch (2011). Calibration curves including isotopically labelled internal standards were used to quantify each hormone concentration using Analyst™ software (PE Sciex, Concord, Ontario, Canada).

2.7. *Statistical analysis*

A two-way mixed ANCOVA of repeated measures was performed, including between (Ramet type) and within factors (Time), using reproductive effort as a covariable. The Tukey test was used as post-hoc method. Data were tested with Shapiro-Wilk and Levene tests for normality and homoscedasticity and transformed whenever necessary. All analyses were performed using the *nlme* and *multcomp* packages in R.

3. Results

3.1. *Physiology of reproductive versus non-reproductive ramets*

The comparison between leaves from reproductive and non-reproductive ramets of *C. edulis* evidenced consistent differences between them in terms of photosynthetic pigments, antioxidants, oxidative damaged molecules and hormonal contents. Leaves from ramets with fruits contained significantly lower total chlorophyll content (**Fig. 2C**), chl *a/b* ratio and total carotenoids (**Fig. 3A-B**). However, the ratio of carotenoids per chlorophylls was found higher in leaves from reproductive ramets (**Fig. 3C**). The ratio of α-tocopherol per chlorophyll unit was found also significantly higher in leaves from reproductive ramets (**Fig. 3F**). Moreover, reproductive ramets leaves contained significantly higher α-tocopherol and lipid hydroperoxides contents (**Fig 3. D-E**). Endogenous cytokinin contents also differed between leaves from different ramet types. The highest contents were found for the most active cytokinin in plants, *tZ*, with leaf concentration around 30 ng g^{-1} (**Fig. 4**). Concentrations of *trans*-zeatin (*tZ*) and its precursor, *trans*-zeatin riboside (*t*ZR) were significantly higher in *C. edulis* reproductive ramets (**Fig. 4 A-B**). No differences between leaves from different ramet types were detected for isopentenyl adenine (2iP) and isopentenyl adenosine (IPA) concentrations (**Fig. 4C**).

When observing the ramets response to drought and recovery, after almost two months with little rain, clear days and high temperatures (**Fig. 1A**), *C. edulis* leaves hydration was found to fall to 7.66 $gH_2O \cdot gDW^{-1}$ ± 0.26 (**Fig. 2A**). After two significant October rains of more than 50 L m^{-2} (**Fig. 1B**), *C. edulis* rehydrated its reproductive and non-reproductive leaves to 9.70 ± 0.25 $gH_2O \cdot gDW^{-1}$ (**Fig. 2A**). No photoinhibition was detected in *C. edulis* leaves during this period, as all F_v/F_m values were found higher than 0.75 (**Fig. 2B**). However, chlorophyll contents decreased during the drought stress period and recovered significantly in leaves from both reproductive and non-reproductive ramets after the September rains (**Fig. 2C**). The chlorophyll recovery was accompanied with an increase in the chl *a/b* ratio (**Fig. 3A**), by a maintenance of the α-toc and lipid hydroperoxides contents, and a significant reduction of the ratio carotenoids and tocopherols per chlorophyll unit (**Fig. 3 C-F**). Finally, *tZ* also increased significantly in regreened reproductive leaves, accumulating 5 times more than in non-reproductive ramets leaves (**Fig. 4B**).

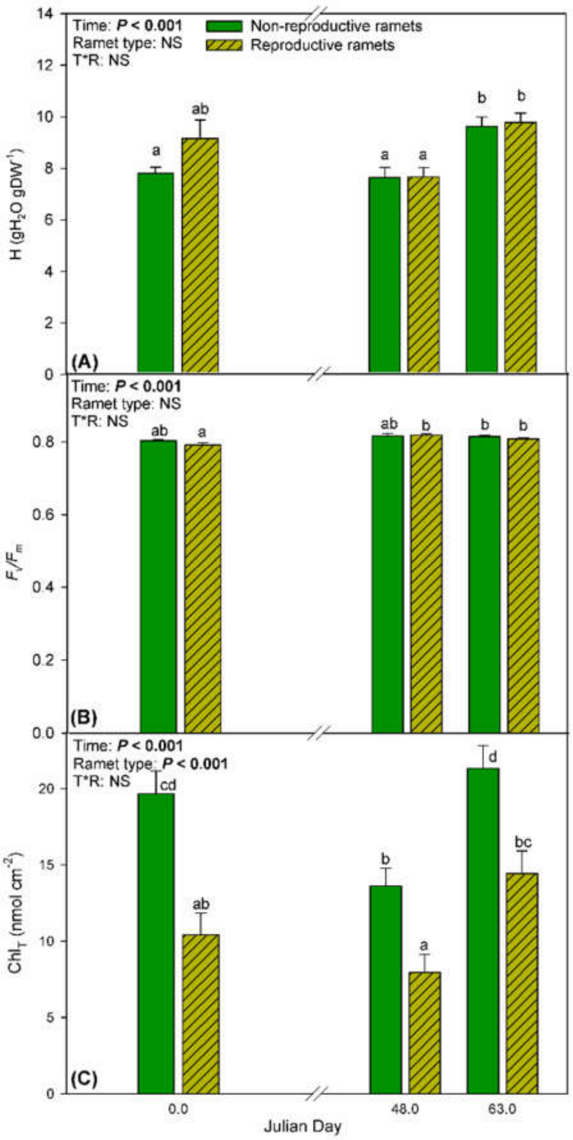


Figure 2. Occurrence of re-greening in *C. edulis* during Mediterranean summer. Leaf hydration **(A)**, maximum quantum efficiency of the photosystem II (F_v/F_m) **(B)** and total chlorophyll content **(C)** in leaves from non-reproductive and reproductive ramets of the invasive species *C. edulis* under natural conditions during Mediterranean summer, after a July rain (Day 0), after 48 days without raining (Day 48) and after significant rains in September (Day 63). Letters correspond to Tukey post-hoc analysis. Data is shown as Mean ± SE (N=20).

Table 1. Re-greening is not promoted by fruit removal in leaves from reproductive ramets. Leaf hydration, maximum quantum efficiency of the photosystem II (F_v/F_m), total chlorophyll content, chlorophyll *a/b* ratio and the two most abundant cytokinins: *trans*-zeatin (*t*Z) and *trans*-zeatin riboside (*t*ZR) in leaves from reproductive ramets (control) and reproductive ramets with fruit removal of the invasive species *C. edulis* under natural conditions during Mediterranean summer, after a July rain (Day 0), after 48 days without raining (Day 48) and after significant rains in September (Day 63). No significant differences within-time were found between leaves from reproductive ramets and leaves from fruit-removed reproductive ramets (*P*-value > 0.05). Data is shown as Mean ± SE (N=20).

Julian Day	Treatment	H ($gH_2O\ gDW^{-1}$)	F_v/F_m	Chl_T (nmol cm^{-2})	Chl *a/b*	*tZ* (ng gDW^{-1})	*tZR* (ng gDW^{-1})
0	**Control**	8.28±0.42	0.79±0.01	9.83±1.41	2.30±0.05	35.26±5.88	10.71±2.66
0	**Fruit removal**	8.47±0.47	0.78±0.01	9.34±0.90	2.31±0.04	47.81±4.29	10.09±2.07
48	**Control**	7.66±0.37	0.82±0.00	7.96±1.18	2.33±0.04	22.37±4.27	13.70±1.50
48	**Fruit removal**	8.02±0.47	0.81±0.00	7.65±0.98	2.27±0.06	44.16±6.39	13.50±1.85
63	**Control**	9.78±0.36	0.81±0.00	14.44±1.49	2.45±0.07	46.32±8.18	13.61±2.93
63	**Fruit removal**	9.89±0.42	0.81±0.00	13.05±1.56	2.60±0.07	60.75±9.00	15.30±2.49

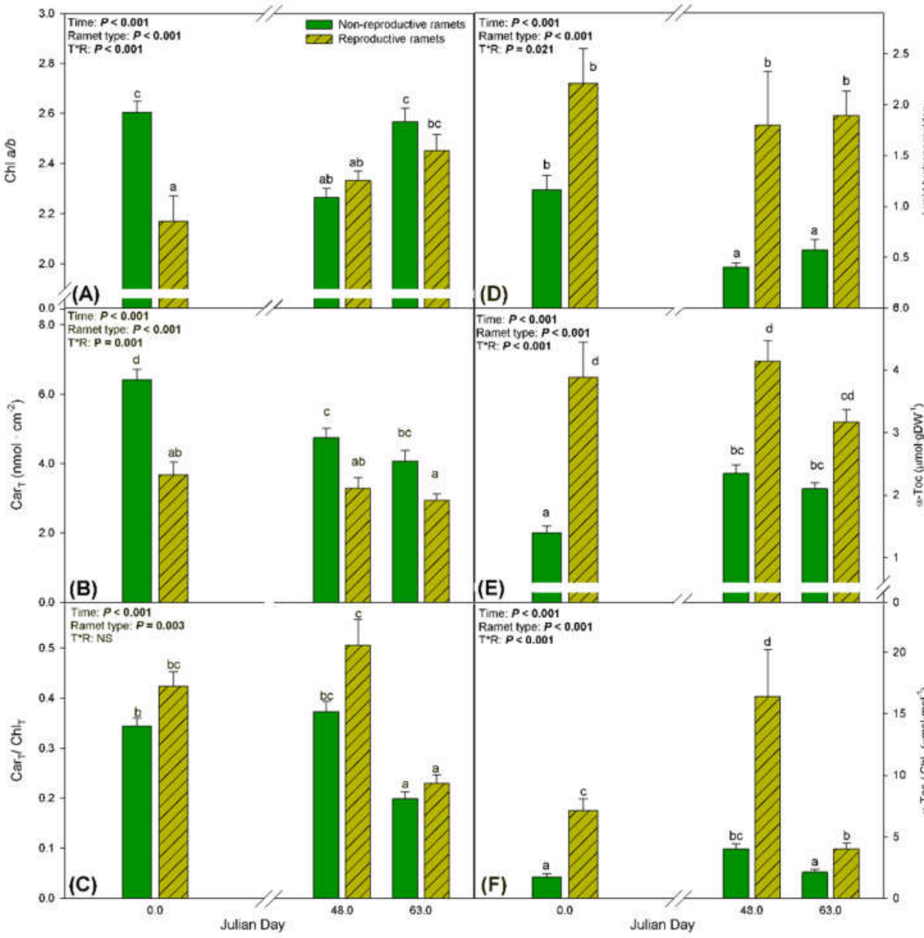


Figure 3. Photoprotection and alleviation of photo-oxidative stress during re-greening. Chlorophyll *a/b* ratio **(A)**, total carotenoids content **(B)**, total carotenoids per chlorophyll unit **(C)**, accumulation of lipid hydroperoxides **(D)**, α-tocopherol content **(E)** and α-tocopherol per chlorophyll unit **(F)** in leaves from reproductive and non-reproductive ramets of the invasive species *C. edulis* under natural conditions during Mediterranean summer, after a July rain (Day 0), after 48 days without raining (Day 48) and after significant rains in September (Day 63). Letters correspond to Tukey post-hoc analysis. Data is shown as Mean ± SE (N=20).

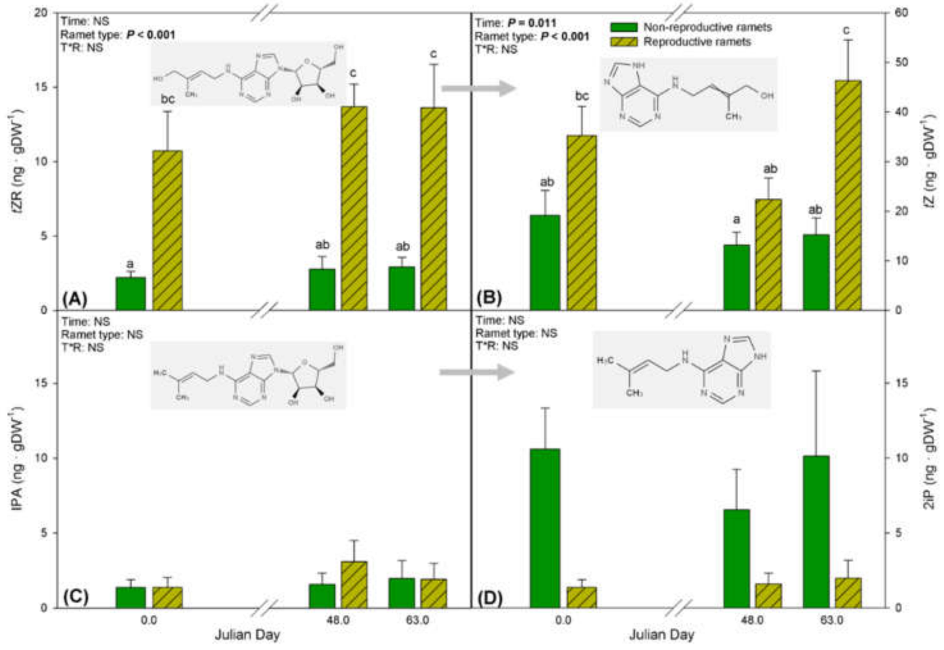


Figure 4. Involvement of cytokinins during re-greening. Endogenous contents of trans-zeatin riboside (tZR) **(A)**, trans-zeatin (tZ) **(B)**, isopentenyl adenosine (IPA) **(C)**, and isopentenyl adenine (2iP) **(D)** in leaves from reproductive and non-reproductive ramets of the invasive species *C. edulis* under natural conditions during Mediterranean summer, after a July rain (Day 0), after 48 days without raining (Day 48) and after significant rains in September (Day 63). Letters correspond to Tukey post-hoc analysis. Data is shown as Mean ± SE (N=20).

3.2. Fruit removal does not affect reproductive ramets physiology

Before the fruit removal treatment (Day 0), the chosen *C. edulis* patches for the fruit removal treatment showed similar (*P*-value > 0.05) hydration, maximum quantum efficiency of the photosystem II, chlorophyll content, chlorophyll *a/b* ratio and cytokinin contents (**Table 1**). The removal of the fruit did not exert any significant alteration in the analyzed parameters at days 48 and 63 (**Table 1**). Similar leaf hydration, maximum quantum efficiency of the photosystem II, total chlorophyll content and chlorophyll *a/b* ratio were observed between leaves from reproductive ramets and leaves from reproductive ramets whose fruit was removed, at the maximum stress point (day 48) and after recovery (day 63). Likewise, no significant differences were found in *t*Z-type and iP-type cytokinins contents or variation during stress and recovery (**Table 1**). Reproductive ramets whose reproductive load was detached behaved identically to control reproductive ramets during the sampling period.

3.3. Patches reproductive effort contribute to stress tolerance and recovery

Reproductive effort in terms of fruit density if *C. edulis* patches ranged from 0.1 to 2.5 Kg fruit m^{-2} (15 to 160 fruits m^{-2}) (**Fig. 5**). Correlation analysis revealed a relationship between reproductive effort and leaf stress markers. For instance, hydration of reproductive and non-reproductive ramets leaves was higher in *C. edulis* patches with higher fruit density ($R^2 = 0.414$, *P*-value < 0.001) (**Fig. 5**). Likewise, the recovery of chlorophyll and total carotenoid content in non-reproductive ramets leaves after stress showed a significant correlation with patches reproductive effort ($R^2 = 0.483$, *P*-value = 0.036 and $R^2 = 0.687$, *P*-value < 0.001 respectively) **(Fig. 5**). Despite the low concentrations found for isopentenyl adenosine (IPA, **Fig. 4C**) strong significant correlations with reproductive effort were identified at the maximum stress point, the hormone loss during the drought stressful period and hormone recovery (**Fig. 5**).

4. Discussion

In this study drought stress and recovery responses of reproductive and non-reproductive ramets of *C. edulis* under natural conditions were evaluated, revealing differential responses among ramet types within individuals that may be associated with the senescing process in monocarpic reproductive ramets and may be regulated by different cytokinins. Environmental induced re-greening, mediated by *trans*-zeatin, occurred under natural conditions in senescing ramets with potential relevant biological significance resuming seed filling regardless periods of adverse environmental conditions that may contribute to species invasive success.

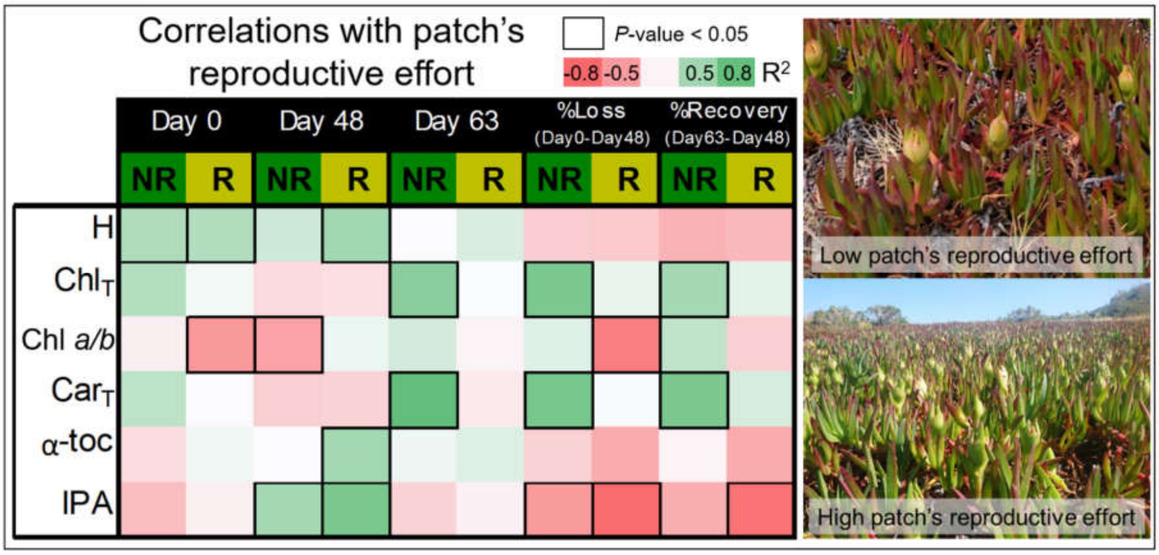


Figure 5. The *C. edulis* patch reproductive effort role on ramet stress and recovery responses. Hydration (H), total chlorophyll content (Chl_T), chlorophyll *a/b* ratio (Chl *a/b*), total carotenoid content (Car_T), α-tocopherol content (α-toc) and isopentenyl adenosine (IPA) showed at least one significant Pearson correlation (framed, *P*-value < 0.05) with *C. edulis* patch reproductive effort (Kg fruit/m^2). Red to green palette reflects correlation coefficient.

4.1. Enhanced photoprotection during reproductive ramet senescence

There are several factors controlling an individual heterogeneity such as developmental status, microclimatic differential conditions or the distance from the reproductive load. In *C. edulis*, the reproductive load distance promoted consistent leaf variation between leaves from reproductive and non-reproductive ramets (with and without fruits respectively). These differences were observed in photoprotective investments but also in cytokinin contents. Leaf senescence in monocarpic plants is associated to the nutrient remobilization process to the fruit to ensure seed survival (Thomas 2013). Chloroplasts, that contain up to 75% of the nitrogen content in photosynthetic tissues (Forde and Steer 1976), are degraded once senescence is induced, leading to a decrease in photosynthetic rates (Wingler et al. 2004). Oxidative damage may eventually occur if antioxidant molecules do not prevent and counterbalance reactive oxygen species (ROS) formation (Huang et al. 2019). Reactive oxygen species may induce lipid peroxidation and promote the accumulation of its primary damaged products, lipid hydroperoxides, that at the same turn may damage photosystems (Pospíšil and Yamamoto 2017). The redox control and homeostasis during plant reproductive development is crucial to ensure the completion of the vital cycle (Zinta et al., 2016). According to this, the consistent increased levels of lipid hydroperoxides that may result from a ROS-induced lipid peroxidation process suggest increased oxidative stress in leaves from reproductive ramets in *C. edulis*. At the same time, the higher photoprotective capacity and antioxidant compounds found in reproductive ramets of *C. edulis* may play an important role during ramet senescence by controlling ROS formation in both space and time, preventing higher oxidative stress. Spatial patterns of photosynthetic and photoprotective processes in senescing tissues of *Arabidopsis thaliana* contribute to prevent premature cell death and to allow efficient nutrient mobilization from senescing leaves (Wingler et al. 2004). Cytokinins regulate various aspects of plant growth and development, including leaf senescence (Lim et al. 2007) and play also a role in ROS accumulation, as ROS homeostasis imbalance was observed in cytokinin-treated plants or mutants with altered cytokinin production or degradation (Mýtinová et al. 2010, Lubovská et al. 2014, Xu et al. 2016). In accordance with this, higher cytokinin contents were observed in senescing *C. edulis* leaves (from reproductive ramets) in the most active (*t*Z), its precursor (*t*ZR) and 2iP, in comparison with leaves from non-reproductive ramets, suggesting that those hormones contribute to control the delicate senescing process in reproductive *C. edulis* ramets during the Mediterranean summers.

4.2. Environmental driven re-greening: insights towards its mechanisms and biological significance

Under natural conditions, with the first big rains after a long drought period, *C. edulis* leaves recover its chlorophyll contents, they re-green after stress. Surprisingly, chlorophyll recovery occurred both in leaves from senescing (reproductive) and non-senescing (non-reproductive) ramets. It is well known that chlorophylls, as light collectors, constitute a central hub regulating chloroplast energy accumulation and respond to a wide variety of stressors (Esteban et al. 2015). Chlorophyll loss or de-greening has been reported under severe stress conditions in water-stress experiments in different species at the Mediterranean Basin. In non-reproductive tissues of *Stipa tenacissima*, the loss up to 50% of the leaf chlorophyll did not lead to leaf senescence but rather constitute a reversible process that allows a decrease in light harvesting reducing over-excitation and photodamage risks during the Mediterranean summers that constitute a temporal dissociation between growth optimal temperature and water availability (Balaguer et al. 2002). Our results show that similarly to some Mediterranean species such as *Lavandula stoechas*, *Rosmarinus officinalis*, *Stipa tenacissima* and *Pistacia lentiscus* (Munné-Bosch and Alegre 2000; Balaguer et al. 2002; Munné-Bosch and Peñuelas 2004), *C. edulis* can reversibly lose a large proportion of its chlorophyll during summer. Not only antenna size but also altered composition is a well-known response to different stresses (Esteban et al. 2015). A lower Chl *a/b* ratio is associated with a reaction center reduction, as Chl *a* is located in both light harvesting complexes and reaction centers, whereas Chl *b* is mainly restricted to light harvesting complexes (Croce and Van Amerongen 2011). *C. edulis* non-reproductive ramets leaves stress response was characterized by a reduced Chl *a/b* ratio, high carotenoids and α-tocopherol per chlorophyll unit revealing the importance of photoprotective and antioxidant compounds in proportion to light harvesting on plant stress responses. De-greening in leaves from reproductive ramets was expected as a result of the senescence process that accompanies fruit maturation as a monocarpic species. Contrarily as expected, chlorophyll loss did not depend on the reproductive load as senescence was not reversed in fruit removed reproductive ramets, suggesting that a point of no return was achieved at the beginning of the fruit maturation process, independently of the sink presence. Diversity in fruit removal effects can be found in scientific literature: no fruit removal effect was found in *Salix alaxensis* but fruit removal increased subsequent growth in *Tripsacum dactyloides*, *Pinguicula alpina* and *Lathyrus vernus* (Obeso 2002).

Drought stress was hypothesized to accelerate the senescence process according to previous results with other species (Yang and Zhan 2006). Instead of an accelerated senescing process, re-greening in senescing tissues of *C. edulis* after recovery was observed. A vigorous growth after a long drought period was observed for transgenic tobacco plants overexpressing an IPT gene, the enzyme that catalyzes the rate-limiting step in cytokinin synthesis (Rivero et al. 2007). The transgenic tobacco plants were able to recover from an imposed drought that killed the control plants, revealing the key role of cytokinins mediating the species drought tolerance and recovery. Moreover, re-greening was observed in cytokinin treated senescent leaves of *Nicotiana rustica* (Zavaleta-Mancera et al. 1999). Although stress intensity modulates increased or decreased cytokinin contents in leaves (Veselov et al. 2017), drought generally causes a reduction of the cytokinins contents which is achieved in *Arabidopsis thaliana* and soybean by repression of IPT genes and the up-regulation of cytokinin oxidase/dehydrogenase enzymes (Le et al. 2012, Nishiyama et al. 2011). Likewise, sharp leaf cytokinin decreases under drought are associated with the operation of the cytokinin oxidase in maize (Brugiere et al. 2003). In *C. edulis*, re-greening was only accompanied by a *t*Z increase suggesting differential roles by the different cytokinins. The *t*Z-type cytokinins are root-derived signals that promote the growth of the above-ground parts of the plant when the rhizosphere is in a suitable condition (Ko and Helarivtta 2017). Whereas iP-type cytokinins are transported through the phloem, *t*ZR, the zeatin precursor, is translocated from the roots through xylem and both *t*ZR content and flow rate of the xylem sap are controlled by environmental signals through root-shoot communication (Takei et al. 2001, Kudo et al. 2010, Ko and Helariutta 2017; Glanz-Idan et al., 2020). The recent study by Mangieri et al. (2020) highlighted the role of *t*Z in delaying leaf senescence in sunflower regarding live root length density. The fact that re-greening in leaves from fruit removed reproductive ramets was also accompanied with an increase in *t*ZR as intact reproductive ramets, suggests that root-shoot communication *via* cytokinins may mediate the observed regreening as a drought tolerance plant strategy in leaves from reproductive ramets in *C. edulis*, rather than a source-sink signaling associated with the monocarpic senescence process (Davies and Gan 2012). The obtained results suggest that the different cytokinins may have different roles in controlling leaf senescence during reproduction, with *t*ZR and 2iP regulating reproductive-associated senescence, and *t*Z controlling chlorophyll recovery (re-greening) in response to environmental cues such as re-watering. In non-reproductive ramets, other mechanisms such as increased cytokinins sensitivity may be mediating the regreening process in *C. edulis*.

The biological significance of the re-greening plasticity in monocarpic species such as *C. edulis* may rely on the potential to optimize seed filling regardless adverse environments. Leaf senescence can be induced by stress to reallocate nutrients to reproductive or young leaves and to eliminate water consumption by older, less productive leaves, resulting into an adaptative stress response (Munné-Bosch and Alegre 2004). The acceleration of developmental senescence in monocarpic species under stressful conditions may be also adaptative, ensuring the completion of the life cycle (Yang and Zhan 2006). However, some studies demonstrated that drought-induced senescence may result in a maladaptive response after severe stresses, impacting seed composition (Sehgal et al. 2018) and seed viability (Su et al. 2013). Additionally, accelerated monocarpic senescence may reduce the chances of seed dispersal in zoochoric species by reducing the time animals may disperse the fruit, as in the case of *C. edulis,* whose fruit is dispersed by little mammals such as rabbits and rats (D'Antonio 1990). In monocarpic species thus, the external regulation of the developmental senescence may potentially offer a plastic strategy following certain environmental cues, allowing species to cope with stressful conditions and resume seed filling when the environmental pressure loose. The ability to re-green after drought stress recovery in *C. edulis* may confer an adaptive advantage to overcome temporal pressures that could compromise seed filling. It is likely that this re-greening capacity is no longer possible at further

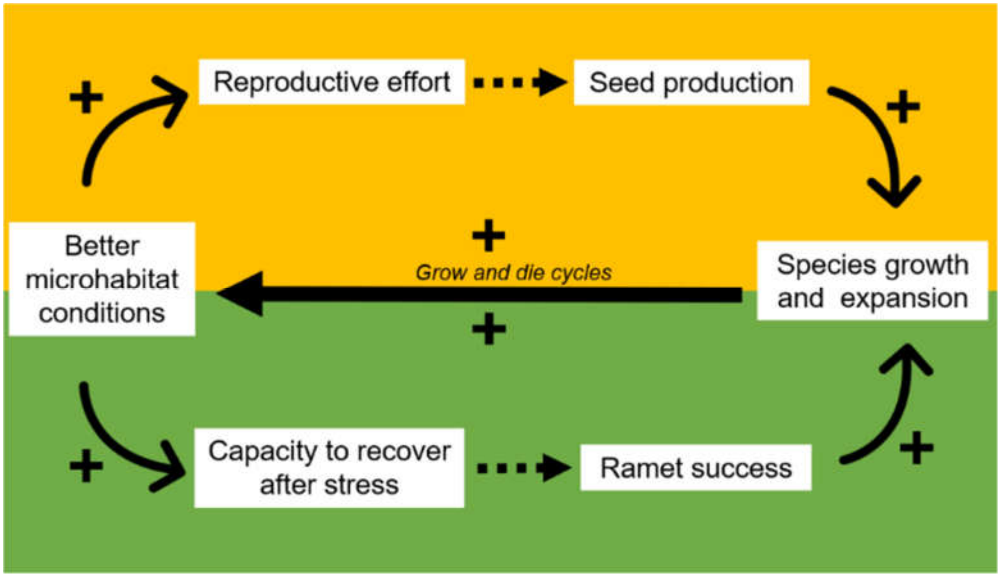


Figure 6. The contribution of reproduction and vegetative growth into the *C. edulis* invasion positive feedback. Proposed scheme considering the obtained results of the correlation between a patch reproductive effort and non-reproductive ramets capacity to recover after stress in relation with the proposed grow and die strategy of this species (Fenollosa et al. 2016) that connect the species reproduction and survival. Yellow and green areas represent respectively sexual and asexual contribution to species growth and expansion.

maturation time, after a point of no return of the senescence process (van Doorn 2005), but it represents a clear advantage for escaping the Mediterranean summer stress.

4.3. Ramet recovery and reproductive effort boost C. edulis invasion positive feedback

In spite of the fact that the relief of the reproductive load did not induce any alteration in the measured parameters in the reproductive ramets of *C. edulis*, some significant correlations were found between the analyzed parameters and the patch reproductive effort in terms of fruit weight per area unit. Besides strong correlation coefficients, correlations do not necessarily suppose causality. The variability of individuals' reproductive effort has been evaluated on different invasive species and have been associated with increased soil nutrient availability. More flowers were produced under low competition and fertilization conditions in the invasive clonal species *Rorippa austriaca* (Dietz et al. 2002). Likewise, lower reproductive effort was found in *Cortaderia selloana* growing at ruderal habitats (Doménech and Vilà 2007). In the specific case of *C. edulis*, Traveset et al. (2008) found a soil determinant role in the reproductive plasticity of the species as flower density in patches of different invaded sites was correlated with different levels of soil nitrogen and pH. Considering this, the correlations found between *C. edulis* patch reproductive effort and hydration, photoprotection and hormonal contents may be an indirect consequence of better microhabitat characteristics. Better microhabitat conditions including higher water retention and nutrient availability may allow higher patch reproductive effort and may play a role in the stress responses and recovery potential of the plant. Indeed, higher reproductive and non-reproductive leaves hydration was found in patches with higher reproductive effort. Non-reproductive ramets from patches with higher reproductive effort recover higher chlorophyll and carotenoid contents after the stress period suggesting that better microhabitat conditions may be promoting ramet success under stressful conditions (**Fig. 6**). Ramet success under stressful conditions may allow vegetative growth and expansion that may induce the enhance of the microhabitat conditions through grow and die cycles as described in Fenollosa et al. (2016). In parallel, the higher reproductive effort at sites with higher microhabitat conditions may result in higher recruitment and also enhance species expansion, contributing also to the grow and die cycle thus defining the *C. edulis* invasion positive feedback (**Fig. 6**). Moreover, higher flower production may enhance the invader impacts as *C. edulis* pollen has been found in native coexistent species interfering native seed production (Jakobsson et al. 2008).Therefore, higher reproductive effort may not only be associated with increased propagule pressure but also enhanced survival species capacity and may be prioritized when managing this invasive species.

5. Conclusions

High plasticity in chlorophyll loss (de-greening) and chlorophyll contents recovery (re-greening) has been observed in the invasive species *C. edulis* in response of Mediterranean drought stress and recovery. Within a *C. edulis* patch, leaves from reproductive ramets showed increased photoprotective investment and cytokinin contents in comparison with leaves from non-reproductive ramets. Regreening plasticity may be regulated *via* tZ-type cytokinins in response to drought stress and may have relevant biological significance resuming seed filling regardless periods of adverse environmental conditions, which may determine species fitness in the frame of climate change. Finally, the found link between patch fruit production and ramet capacity to recover after drought stress may boost *C. edulis* invasion positive feedback with enhanced vegetative growth and reproductive output.

Author contributions

EF and SMB conceived the idea and experimental design. EF performed the data collection, samples analysis and data analysis. EF and SMB contributed to the interpretation of the results. EF prepared the manuscript. SMB revised the manuscript.

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References

Amaral JS, Casal S, Torres D, Seabra RM, Oliveira BPP (2005) Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method. Anal Sci 21: 1545–1548

Balaguer L, Pugnaire FI, Martínez-Ferri E, Armas C, Valladares F, Manrique E (2002) Ecophysiological significance of chlorophyll loss and reduced photochemical efficiency under extreme aridity in *Stipa tenacissima* L. Plant Soil 240: 343–352

Brugiere N, Jiao S, Hantke S, Zinselmeier C, Roessler JA, Niu X, Jones RJ, Habben, JE (2003) Cytokinin oxidase gene expression in maize is localized to the vasculature, and is induced by CKs, abscisic acid, and abiotic stress. Plant Physiol 132: 1228–1240

Cardoso AA, Batz TA, McAdam, SAM (2020) Xylem embolism resistance determines leaf mortality during drought in Persea americana. Plant Physiol 182(1): 547-554

Chenot J, Affre L, Passetti A, Buisson E (2014) Consequences of iceplant (Carpobrotus) invasion on the vegetation and seed bank structure on a Mediterranean island: response elements for their local eradication. Acta Bot Gallica 161: 301-308

Christensen JH, Christensen OB (2007) A summary of the PRUDENCE model projections of changes in European climate by the end of this century. Climatic Change 81:7–30

Croce R, Van Amerongen H (2011) Light-harvesting and structural organization of Photosystem II: From individual complexes to thylakoid membrane. J Photoch Photobio B 104:142–153

D'Antonio CM (1990) Seed production and dispersal in the non-native, invasive succulent *Carpobrotus edulis* (Aizoaceae) in coastal strand communities of central California. J Appl Ecol 27: 693-702

Davies PJ, Gan S (2012) Towards an integrated view of monocarpic plant senescence. Russ J Plant Physl 59:467–478

DeLong JM, Prange RK, Hodges DM, Forne, CF, Bishop MC, Quilliam M (2002) Using a modified ferrous oxidation−xylenol orange (FOX) assay for detection of lipid hydroperoxides in plant tissue. J Agr Food Chem 50: 248-254

Dietz H, Köhler A, Ullmann I (2002) Regeneration growth of the invasive clonal forb *Rorippa austriaca* (Brassicaceae) in relation to fertilization and interspecific competition. Plant Ecol 158:171–182

Domènech R, Vilà M (2007) *Cortaderia selloana* invasion across a Mediterranean coastal strip. Acta Oecol 32: 255–261

Esteban R, Barrutia O, Artetxe U, Fernández-Marín B, Hernández A, García-Plazaola JI (2015) Internal and external factors affecting photosynthetic pigment composition in plants: a metaanalytical approach. New Phytol 206: 268–280

Fenollosa E, Munné-Bosch S, Pintó-Marijuan M (2017) Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*. Physiol Plantarum 160: 185–200

Fenollosa E, Roach DA, Munné-Bosch S (2016) Death and plasticity in clones influence invasion success. Trends Plant Sci 21: 551–553

Fernández-Marín B, Hernández A, Garcia-Plazaola JI, Esteban R, Míguez F, Artetxe U, Gómez-Sagasti MT (2017) Photoprotective strategies of Mediterranean plants in relation to morphological traits and natural environmental pressure: A meta-analytical approach. Front Plant Sci 8: 1-16

Forde J, Steer M (1976) The use of quantitative electron microscopy in the study of lipid composition of membranes. J Exp Bot 27: 1137–1141

Glanz-Idan N, Tarkowski P, Turečková V, Wolf S (2020) Root–shoot communication in tomato plants: cytokinin as a signal molecule modulating leaf photosynthetic activity. J Exp Bot 71: 247-257

Herrera CM (2017) The ecology of subindividual variability in plants: patterns, processes, and prospects. Web Ecol 17: 51–64

Huang H, Ullah F, Zhou DX, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci 10: 1-10

Jakobsson A, Padrón B, Traveset A (2008) Pollen transfer from invasive *Carpobrotus* spp. to natives – A study of pollinator behaviour and reproduction success. Biol Conserv 141: 136-145

Ko D, Helariutta Y (2017) Shoot-root communication in flowering plants. Curr Biol 27: R973–R978

Köppen W (1923) Die Klimate der Erde, Bornträger, Berlin

Krieger-Liszkay A (2005) Singlet oxygen production in photosynthesis. J Exp Bot 56: 337–346

Kudo T, Kiba T, Sakakibara H (2010) Metabolism and long-distance translocation of cytokinins. J Integr Plant Biol 52: 53–60

Kyparissis A, Petropoulou Y, Manetas Y (1995) Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. J Exp Bot 46: 1825–1831

Le DT, Nishiyama R, Watanabe Y, Vankova R, Tanaka M, Seki M, Ham LH, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS (2012) Identification and expression analysis of cytokinin metabolic genes in soybean under normal and drought conditions in relation to cytokinin levels. PLoS One, 7: e42411

Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem Soc Trans 11: 591–592

Lim PO, Kim HJ, Nam HG (2007) Leaf senescence. Annu Rev Plant Biol 58: 115–136

Lubovská Z, Dobrá J, Štorchová H, Wilhelmová N, Vanková R (2014) Cytokinin oxidase/dehydrogenase overexpression modifies antioxidant defense against heat, drought and their combination in *Nicotiana tabacum* plants. J Plant Physiol 171: 1625–1633

Mangieri MA, Hall AJ, Chimenti, CA (2020) Cytokinin dynamics in xylem sap and leaves of fruiting and de-fruited sunflower hybrids of contrasting post-anthesis canopy senescence patterns during flowering and grain-filling. Plant Soil 448: 165-181

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178: 719-739

Mitrakos K (1980) A theory for Mediterranean plant life. Acta Oecol Oecol Plant 1: 234–252.

Müller M, Munné-Bosch S (2011) Rapid and sensitive hormonal profiling of complex plant samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Plant Methods 7: 37

Munné-Bosch S, Alegre L (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210: 925–931

Munné-Bosch S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. Funct Plant Biol 31: 203–216

Munné-Bosch S, Peñuelas J (2004) Photo- and antioxidative protection during summer leaf senescence in *Pistacia lentiscus* L. grown under Mediterranean field conditions. Ann Bot 92: 385–391

Mýtinová Z, Motyka V, Haisel D, Gaudinová A, Lubovská Z, Wilhelmová N (2010) Effect of abiotic stresses on the activity of antioxidative enzymes and contents of phytohormones in wild type and AtCKX2 transgenic tobacco plants. Biol Plant 54: 461–470

Nishiyama R, Watanabe Y, Fujita Y, Le DT, Kojima M, Werner T, Vankova R, Yamaguchi-Shinozaki K, Shinozaki K, Kakimoto T, Sakakibara H, Schmülling T, Tran LS (2011) Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis. Plant Cell 23: 2169–2183

Nooden LD (1988) Whole plant senescence. In: Noodén LD, Leopold AC (ed) Senescence and aging in plants. Academic Press, San Diego, pp 391–440

Obeso JR (2002). The costs of reproduction in plants. New Phytol 155: 321-348

Pospíšil P, Yamamoto Y (2017) Damage to photosystem II by lipid peroxidation products. Biochim Biophys Acta 1861: 457-466.Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E (2007). Delayed leaf senescence induces extreme drought tolerance in a flowering plant. PNAS 104: 19631–19636

Robakowski P, Pers-Kamczyc E, Ratajczak E, Thomas PA, Ye Z-P, Rabska M, Iszkuło G (2018) Photochemistry and antioxidative capacity of female and male *Taxus baccata* L. acclimated to different nutritional environments. Front Plant Sci 9: 1-13

Roiloa S (2019) Clonal traits and plant invasiveness: The case of *Carpobrotus* N.E.Br. (Aizoaceae). Perspect Plant Ecol 40: 1-7

Sehgal A, Sita K, Siddique KHM, Kumar R, Bhogireddy S, Varshney RK, HanumanthaRao B, Nair RM, Prasad PVV, Nayyar H (2018) Drought or/and heat-stress effects on seed filling in food crops: impacts on functional biochemistry, seed yields, and nutritional quality. Front Plant Sci 9: 1-19

Su Z, Ma X., Guo H, Sukiran NL, Guo B, Assmann SM, Ma H (2013) Flower development under drought stress: morphological and transcriptomic analyses reveal acute responses and long-term acclimation in Arabidopsis. Plant Cell 25: 3785–3807

Takahashi S, Badger MR (2011) Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci 16: 53–60

Takei K, Sakakibara H, Taniguchi M, Sugiyama T (2001) Nitrogen-dependent accumulation of cytokinins in root and the translocation to leaf: implication of cytokinin species that induces gene expression of maize response regulator. Plant Cell Physiol 42: 85–93

Thomas H (2013) Senescence, ageing and death of the whole plant. New Phytol 197: 696–711.

Traveset A, Moragues E, Valladares F (2008) Spreading of the invasive *Carpobrotus* spp. in Mediterranean ecosystems: the advantage of performing well in different light environments. Appl Veg Sci 11: 45–54

van Doorn WG (2005) Plant programmed cell death and the point of no return. Trends Plant Sci 10: 478–483

Veselov DS, Kudoyarova GR, Kudryakova NV, Kunetsov VV (2017) Role of cytokinins in stress resistance of plants. Russ J Plant Physl 64: 15–27

Vilà M, Valladares F, Traveset A, Santamaría L, Castro P (eds) (2008) Invasiones Biológicas. CSIC, Madrid.

Wingler A, Marès M, Pourtau N (2004) Spatial patterns and metabolic regulation of photosynthetic parameters during leaf senescence. New Phytol 161: 781–789

Wingler A, Purdy S, MacLean JA, Pourtau N (2006) The role of sugars in integrating environmental signals during the regulation of leaf senescence. J Exp Bot 56: 391–399

Xu Y, Burgess P, Zhang X, Huang B (2016) Enhancing cytokinin synthesis by overexpressing IPT alleviated drought inhibition of root growth through activating ROS-scavenging systems in *Agrostis stolonifera*. J Exp Bot 67: 1979–1992

Yang J Zhang J (2006) Grain filling of cereals under soil drying. New Phytol 169: 223-236

Zavaleta-Mancera HA, Thomas BJ, Thomas H, Scott IM (1999) Regreening of senescent Nicotiana leaves: II. Redifferentiation of plastids. J Exp Bot 50: 1683–1689

Zimmermann P, Zentgraf U (2005) The correlation between oxidative stress and senescence during plant development. Cell Mol Biol 10: 515–534

Zinta G, Khan A, AbdElgawad H, Verma V and Srivastava AK (2016) Unveiling the Redox Control of Plant Reproductive Development during Abiotic Stress. Front. Plant Sci. 7:700

Zwack PJ, Rashotte AM (2013) Cytokinin inhibition of leaf senescence. Plant Signal Behav 8: e24737

Capítol 3. La variabilitat intraespecífica en llavors de l'espècie invasora *Carpobrotus edulis* a diferents escales geogràfiques en l'adaptació al Mediterrani

Chapter 3. The nearest is not the closest: intraspecific variation in seed traits across diferent geographic scales may play a role in local adaptation of the invasive species *Carpobrotus edulis* to Mediterranean-type ecosystems.

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The nearest is not the closest: intraspecific variation in seed traits across different geographic scales may play a role in local adaptation of the invasive species *Carpobrotus edulis* to Mediterranean-type ecosystems

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Abstract

Background and Aims: As essential multidimensional travellers (across space and time), seeds play a major role in plant species persistence and expansion. Consequently, seed traits are subjected to strong evolutionary pressures contributing to plant local adaptation and evolution, and therefore strong variability may be expected. However, the magnitude of those effects across the geographic space may be underestimated. The aim of this study is to evaluate the existence of intraspecific variability in seed traits of the invasive species *Carpobrotus edulis* at different geographical scales, contrasting closer and distant populations. As a novel ecosystem player, this invader may be highly affected by heterogeneous environmental pressures across the landscape.

Methods: More than 30 parameters were measured to evaluate seed production, morphology, vigour and longevity of nine populations of *C. edulis* along the Catalan coast (NE Spain) from three differentiated zones with a disturbance gradient. Geographic and environmental distances between populations were calculated and contrasted against individual seed traits distances and multivariate distance to explore the spatial trait variation along the territory and the environmental influence.

Key Results: The analysis revealed high intraspecific variability among populations regarding seed size, mass, viability, germination rates and longevity, that was not explained by geographic distance, as regardless the little distance between some populations (< 0.5km), significant differences were found in seed morphology, viability, germination, dormancy and longevity. Seed production, viability, germination and persistence traits showed the strongest spatial variability (trait variability with geographic distance), but this was not constant within the three analysed zones. Increased seed traits variability was found in the zones with increased human presence. Moreover, seed traits variability correlated with bioclimatic variables towards increased variability in colder and drier conditions.

Conclusions: The measured traits have strong variation with geographic distance, leading to differentiated *C. edulis* soil seed bank dynamics. Thus, differential strategies may be required for a cost-effective management of this species considering different populations. Seed trait variability is influenced by disturbance but also environmental conditions, suggesting a potential impact of increased anthropogenic pressure and climate shifts. Such high intraspecific variability may play a major role in increasing plasticity in natural seed banks and may it turn facilitate local adaptation to environmental stressors, aggravating invasive impacts.

Keywords (3-12): Bioclimatic variables, *Carpobrotus edulis* (L.) N. E. Br., Distance, Dormancy, Heterogeneity, Intraspecific trait variation, Invasive species, Management, Mediterranean, Plasticity, Seed traits, Soil seed bank.

Introduction

Determination of invasive species persistence and expansion potential is crucial to understand their success, quantify their impact on native communities and develop cost-effective management strategies (Vilà *et al.*, 2011). Seeds constitute one of the main factors defining the persistence and expansion of species and therefore have the potential to play a crucial role determining invasive success (Gioria and Pyšek, 2016). As essential multidimensional travellers across space and time, seeds possess the ability to determine population recruitment and dispersion towards new territories. Indeed, some plant traits associated with sexual reproduction such as high seed production, small seed mass and rapid germination have been associated with higher invasive success (Pyšek and Richardson 2007; Gioria and Pyšek 2017; Gioria *et al.* 2018). Variations in seed morphological traits may determine differential dispersion strategies that can favour adaptation and colonisation of different habitats by invasive species (Lamberti-Raverot *et al.*, 2019). Likewise, high viability and earlier germination may explain high invasion potential of the invasive species *Eragrostis plana* in Brazil (Guido *et al.*, 2017) and dispersal ability in terms of seed mass and plume loading was selected during range expansion of the invader *Mikania micrantha* (Huang et al., 2015)

Some successful invaders also benefit from the ability to develop a persistent soil seed bank (Passos *et al.*, 2017). In species with orthodox seeds, viable seeds may enter dormancy, a temporal suppression of germination under the conditions favourable to germination thanks to their ability to tolerate considerable desiccation (Bewley *et al.*, 2013). Complex hormonal regulation prevents seeds to germinate, resisting for several years at the soil seed bank (Kucera *et al.*, 2005). Different seed longevities have been described for several species, ranging from just a few months to more than 2,000 years in *Phoenix dactylifera* (Sallon *et al.* 2008). Even within a species, seed longevity may vary based on several factors such as moisture content, relative humidity, oxygen pressure and temperature (Walters *et al.*, 2005). During ageing, seeds are subjected to oxidative stress that may be counterbalanced with antioxidants compromising seed longevity (Bailly, 2004). Among antioxidant systems, tocochromanols are lipophilic antioxidants particularly abundant in seeds that were found to be essential determining seed longevity (Sattler *et al.*, 2004). Due to the difficulties to measure seed longevity under natural conditions, accelerated ageing tests are used to estimate it (Hay *et al.*, 2019). The characterization of seed production, viability, longevity and germination may offer an approximated reflect of the invasive soil seed bank dynamics (Gioria and Pyšek, 2016).

Despite all well-known seed processes and the role of seeds on invasive species persistence and expansion, the magnitude of seed production, viability, germination and persistence intraspecific variation in the territory may be underestimated. Indeed, Jarić *et al.*, (2019) proposes a conceptual framework of crypticity in biological invasions that include spatial variation in seed traits as one of the contributors to the uncertainty when trying to model invasive species impact or develop management strategies. Intraspecific variation of seed traits constitutes a major concern in the research agenda for seed-trait functional ecology (Saatkamp et al., 2018) as the reliability of trait-based mechanistic models depends on the representation of intraspecific trait variation, that may drive to enormous differences (Albert et al., 2011). In invasive species, strong spatial variation in seed traits will lead to differential interactions with native species and variability on invasive impacts that may require differential management strategies (Januchowski-Hartley *et al*., 2017). Seed traits variability may come from different sources such as maternal environment, seed level variability (due do differential seed environment), cell level variability (due to transcription, translation, degradation, cellular environment, uneven portioning, etc.) and seed developmental variability (due to differences in developmental switches) (Mitchell *et al*., 2017) and may contribute to invader's geographical crypticity.

High phenotypic plasticity has been observed in the invasive species *Carpobrotus edulis* (L.) N. E. Br. regarding biomass allocation, morphological variations and photoprotective responses (Traveset, Moragues and Valladares, 2008; Roiloa *et al*. 2014; Roiloa *et al*. 2016; Fenollosa *et al*., 2017). *C. edulis* (Aizoaceae) is a mat-forming trailing succulent perennial native from South Africa that has been introduced in all continents, strongly impacting Mediterranean regions (Vilà *et al.,* 2008). *C. edulis* was introduced in Europe as a valuable ornamental plant but also for soil and dune stabilization (Preston and Sell, 1988). *C. edulis* impacts reside on its ecosystem engineer capacity to modify the surrounding environment by altering physicochemical soil properties (Molinari et al., 2007, Novoa et al., 2013) and decrease native plant richness and functional diversity (Jucker et al., 2013 Novoa and González, 2014). *C. edulis* has small hard-coated reniform orthodox seeds, which can generate a permanent soil seed bank that may contribute to the species impact and persistence in the invaded communities (Chenot *et al*., 2014; Fenollosa et al., 2020). *Carpobrotus sp.* seeds may persist in the soil seed bank for more than 5 years allowing quick reinvasion many years after removal (up to 8 years) (Affre 2011; Ruffino et al. 2015). However, the magnitude of seed traits variability across different geographic scales has not been studied and may determine differential invasive vigour in this highly plastic invasive species.

The aims of this study were to (a) evaluate the existence of intraspecific variability in plant traits associated with sexual reproduction of the invasive species *C. edulis* at different geographical scales, contrasting closer (< 4km) and distant (> 40km) populations and (b) evaluate differences in intraspecific trait variability across the three zones with a disturbance gradient. We expected high intraspecific variability in *C. edulis* seed traits across the territory due to the strong evolutionary pressures a novel ecosystem player may be subjected to in a heterogeneous landscape. We also expected seed traits dissimilarity to be associated by geographic distance, with closer populations being more similar than distant ones, revealing differences in invasive potential. Finally, we expected increased seed trait variability in zones with increased disturbance that may generate different microhabitat conditions. This information may be helpful to understand seed traits variability across an invaded area and to develop efficient management strategies.

Material and Methods

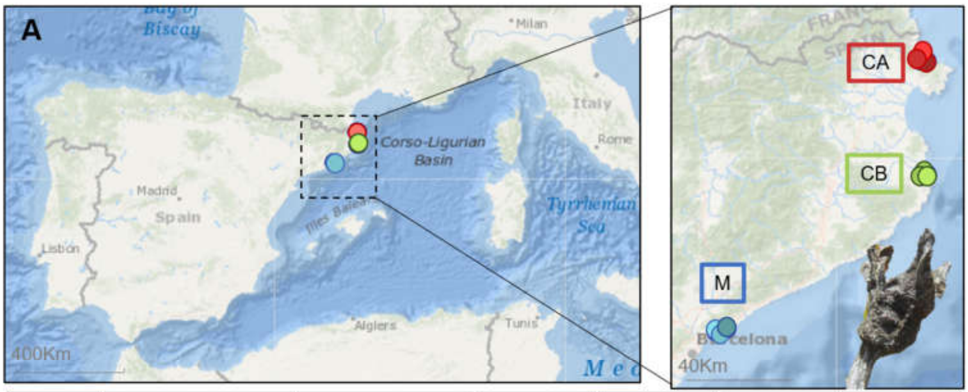
Plant populations and seed collection

Nine populations of *C. edulis* along the Catalan coast (NE Spain) were selected considering different geographic distances. Within three distant zones (> 40km) (Cap de Creus, Costa Brava and Maresme), three populations were sampled based on closeness (< 4km) and enough fruit production to measure all seed traits (**Figure 1**). In Catalonia, *Carpobrotus edulis* inhabits most of the coastal zones. However, the shoreline has been massively urbanized, leaving few spaces for vegetation. Across the Catalan coast some zones have been urbanized more than others. In the northern part, the zone of Cap de Creus (CA) is the eastern foothill of the Pyrenees. It includes marine and terrestrial protected areas and constitute an area of high biological, geological and landscape quality with large extensions of woody sclerophyll Mediterranean vegetation. Forty kilometres south, the zone of Costa Brava constitutes an area where tourism is more intense and combine large zones of established plant communities coexisting with urbanizations, campings and touristic complexes. Finally, getting closer to Barcelona, the Maresme (M) coast is intensely urbanized with large agricultural areas leaving almost no virgin plant communities. Among those three zones there is a strong gradient in human presence in terms of population density per squared kilometre. In average, the three *C. edulis* populations in the CA zone have a population density of 39 habitants km^{-2}, whereas CB has 188 habitants km^{-2} and M 285 habitants km^{-2} (Geostat, Eurostat, 2011). These differences in human presence and habitat availability also influence the surrounding vegetation, besides terrain characteristics. According to Carrillo et al. vegetation cartography (2018), the *C. edulis* populations in the less human populated zone, CA occur within the following vegetation units: cliffs colonized by *Armeria ruscinonens*is or *Plantago subulata*, Mediterranean lavender maquis and xerophilus silicic grassland with *Lupino-Lavanduletum stoechadis*, *Trifolio-Brachypodietum ramosi, Helianthemion guttati* + *Hyparrhenientum* + *Trifolio-Brachhypodietum ramosi*. The CB populations are found within maritime Aleppo pine forests with a calcicolous scrub layer of *Rosmarino-Ericion,* coastal wooded dunes of *Pinus pinea* or *P. halepensis* with a heliophile scrub layer and coastal white dunes, dominated, when vegetated by *Ammophiletum arundinaceae*, *Agropyretum mediterraneum* and *Crucianelletum maritimae*. Finally, the M zone *C. edulis* populations fall in *Panico-Setarion* crops, fallow land and waste places with *Echio-Galactition*, *Bromo-Oryzopsion* and urban areas. Unfortunately, there is no data regarding introduction time or invasion front within the territory.

Climatic data was obtained from the WorldClim 2 database at 0.5 arcmin resolution (~1 km^2) (Fick and Hijmans, 2017). Environmental and geographic distances between populations were estimated using the *dist()* function in R 3.5.0. From each population, within the period of natural seed rain, 600 fruits were collected to ensure representativeness. Fruits were opened and all the obtained seeds were pooled (more than 10.000 seeds per population). Seeds were kept at darkness and room temperature until analysis (3-4 weeks). A total of ecological significant 39 traits related with seed production, seed morphology, viability, germination, persistence and biochemistry were measured and are summarized in **Table 1**.

Seed morphology and production

Five fruits from each population were used to determine seed production in terms of fruit weight (FruitWeight), total seed weight (SeedsWeight) and total seed number (SeedNum). From each fruit, five seeds were randomly selected to measure morphometric seed parameters such as seed area, perimeter, major diagonal, minor diagonal and thickness, considering basal and lateral scaled captures of 4x magnification under a binocular loupe leading to the following parameters from basal capture: basal seed area (AreaB), basal seed perimeter (PerimB), major basal diagonal (MajorB), minor basal diagonal (MinorB), and from lateral capture: lateral seed area (AreaL), lateral seed perimeter (PerimL), major lateral diagonal (MajorL), minor lateral diagonal (MinorL). All measurements were performed with ImageJ software (Wayne Rasband, Java, National Institutes of Health, Bethesda, USA). Five replicates of fifty seeds were used to estimate seed mass (SeedMass) and the dry seed mass (DrySeedMass) after four days at 70ºC. Seed mass and dry seed mass were obtained by dividing the obtained fresh and dry weight respectively by seed number.



Code	Population	Zone	Lon	Lat	T (ºC)	P (mm)
CA1	Far de s'Arenella	Cap de Creus	3.186813	42.350243	15.23	530
CA2	Punta de la Creu	Cap de Creus	3.204676	42.346923	15.33	510
CA3	Punta del Podaire	Cap de Creus	3.177721	42.35352	15.42	528
CB1	Platja de Pals	Costa Brava	3.204351	41.983539	15.38	493
CB2	Carrer del Golf	Costa Brava	3.199613	41.986519	15.41	495
CB3	Puig Sa Guilla	Costa Brava	3.193844	41.984878	15.35	499
M1	Ca l'Antic	Maresme	2.29852	41.473742	15.73	585
M2	Can Teixidor	Maresme	2.30128	41.475079	15.78	587
M3	Camí del Mig	Maresme	2.337122	41.484476	15.83	594

B

Geographic distance (km)

	CA1	CA2	CA3	CB1	CB2	CB3	M1	M2	M3
CA2	1.5								
CA3	0.8	2.3							
CB1	40.8	40.4	41.2						
CB2	40.4	40.0	40.8	0.5					
CB3	40.6	40.2	41.0	0.9	0.5				
M1	122.1	122.7	121.9	94.3	94.1	93.7			
M2	121.8	122.5	121.7	94.0	93.9	93.4	0.3		
M3	119.2	119.8	119.1	91.0	90.9	90.4	3.4	3.2	

Environmental distance

	CA1	CA2	CA3	CB1	CB2	CB3	M1	M2	M3
CA2	2.6								
CA3	3.0	1.6							
CB1	7.3	6.8	6.5						
CB2	5.9	5.2	5.5	3.4					
CB3	6.5	5.8	6.1	3.6	1.5				
M1	7.0	6.2	5.9	7.6	7.4	7.7			
M2	7.2	6.4	5.9	8.1	7.8	8.1	1.3		
M3	7.7	7.0	6.5	8.2	8.2	8.4	2.3	1.4	

Figure 1. Localization, environmental conditions and geographic and environmental distances of the 9 *Carpobrotus edulis* selected populations along the three zones at Catalan coast: Maresme, Costa Brava and Cap de Creus. In the geographic and environmental matrices, a grayscale palette is used to reflect data difference.

Seed vigour: Germination, viability and longevity

Seed germination for all populations was performed as described in Fenollosa et al. (2020) with 12h photoperiod and temperature alternance between 25ºC during day and 10ºC at night. Germination was assessed every three days on petri plaques with fifty seeds each with five replications per population. Total germination percentage (GRP) was estimated after curve saturation at 70 days. Different seed germination indexes regarding speed and synchrony were estimated: mean germination time (MGT) (days), mean germination rate (MGR) (seeds day^{-1}), germination speed percentage (GSP) (%), uncertainty index (UNC) (bits), synchronization index (SYN) (from 0 to 1), germination variance (VGT), germination standard deviation (SDG), coefficient of variation (CVG) (%), and T_{50} (The time required for 50% germination). The germination uncertainty index (UNC) is based on the Shannon indexes and estimates the uncertainty in predicting the informal entropy associated with the distribution of the germination relative frequency in bits units (information units) (Labouriau and Valadares, 1983). The synchronization index (SYN) assesses the synchrony of one seed with other included in the same replication (SYN = 0 when at least two seeds germinate one each time and SYN =1 when germination of all the seeds occurs at the same time) (Ranal and Santana, 2006). Five replications per population, including fifty seeds per replication were used for germination test.

Seed viability was assessed using the viability tetrazolium (Triphenyl tetrazolium chloride) test following the method described in Fenollosa et al. (2020). After 24h of seed imbibition, seeds were delicately pierced to ensure tetrazolium penetration. Seeds with 0.1% tetrazolium (Sigma-Aldrich, Steinheim, Germany) were incubated for 48h at 40ºC before viability assessment. Six different categories were used to classify embryo status: viable (totally stained), weakly (pale stained), patchy (partially stained), patchy-weakly (partially pale stained), dead (white embryo), and aborted (no embryo found). The sum of weakly, patchy and patchy-weakly seeds constitutes low vigour seeds without survivance probabilities (Dying). Percentage of dormant seeds was estimated as the difference between viable but not germinated seeds.

Seed longevity was assessed through an accelerated ageing test as described by Fenollosa et al. (2020) on which seeds were subjected to 55ºC under high relative humidity conditions (80-90%) for different timings that lead to a progressive viability loss. Viability was totally loss for all populations after 196h under the accelerated ageing conditions and L_5 (resistance to deterioration), L_{50} (medium longevity) and L_{95} (lethal ageing time) (Number of hours to lose 5, 50 and 95% viability respectively) was used as longevity estimators. Five replicates per population, including 250 seeds per replication were used for viability and longevity assessment.

Seed biochemistry: antioxidants and seed water content

Tocochromanols are lipid-soluble antioxidant molecules and its accumulation in seeds has been described to be critical in maintaining seed viability by protecting lipids from oxidation during germination and early seedling growth (Sattler et al. 2004).Five replicates of 100mg of seed samples from each population were ground in liquid nitrogen using a mix ball and extracted with cold methanol containing 0.01% butylated hydroxyltoluene using ultrasonication. After centrifuging at 14,000 *g* for 10 min at 4ºC, the supernatant was collected and the pellet re-extracted with the same solvent until it was colourless; then, supernatants were pooled and filtered with 0.22 µm and transferred to high-performance liquid chromatography (HPLC) vials. Tocochromanols were separated isocratically in a normal-phase HPLC system and quantified with a fluorescent detector, as described by Amaral *et al.* (2005). Quantification was based on the results obtained from the fluorescence signal and compared to that of a calibration curve made with authentic standards (Sigma-Aldrich, Steinheim, Germany). Gamma-tocopherol (γ-Toc), alpha-tocopherol (α-Toc) and their sum (Toc) were detected in *C. edulis* seeds.

Seed water content (WC), and imbibed seed water content (WC_Imb) was measured with five replicates of fifty seeds per population as (fresh - dry weight*100) * dry weight^{-1} from seeds and imbibed seeds for 24h respectively. Dry weight was obtained after four days at 70ºC.

Data analysis

Germination parameters were calculated using *GerminaR* package. A nested ANOVA was used to evaluate thesignificance of the factors Zone and Population (nested in Zone). Data were tested with Shapiro-Wilk and Levene tests for normality and homoscedasticity. Permutational multivariate analysis of variance (PERMANOVA) was used to contrast zones and populations (nested in zone) using a multidimensional approach with 999 permutations with scaled data, and Mantel tests were run to evaluate correlation between distance matrices using the *vegan* R package. To evaluate the correlation of seed traits and bioclimatic variables to the multivariate populations differentiation, the *envfit()* function from the *vegan* R package was used. As measured traits resulted into independent replicates, PERMANOVA results were tested after multiple dataset randomizations to confirm the obtained statistic results. To assess the intraspecific traits variability within a zone, the *betadisper()* function from the *vegan* R package was used. To evaluate the variation of a trait with the geographic distance, percentual trait change was calculated for all populations, evaluating the percentual difference of each individual point to all the others an its geographic distance. Correlations were tested using the *cor()* function in R. The Tukey test was used as a post-hoc method using the *multcomp* and *agricola* packages. The *ggplot2* package was used for plotting. All analyses were performed in R 3.5.0.

Results

Near populations differ significantly

When contrasting the coordination between geographic distance and environment variables at the studied populations across the Catalan coast, a strong correlation between geographic and environmental distances was revealed (R_M = 0.787, P = 0.002) (see distance matrices in **Figure 1B**). The three geographically distant zones (Cap de Creus, Costa Brava and Maresme) showed an annual precipitation from 490 to almost 600 mm and an annual mean temperature around the 15ºC (**Figure 1A**).Significant differences between populations were found considering almost all measured seed parameters when contrasting both distant and near populations (**Figure 2, Table S1**). The multivariate analysis of the populations revealed significant effects for zones and populations ($P < 0.001$) for seed morphology, production, germination, biochemistry and persistence trait types (**Figure 2**). Moreover, almost all variables showed a strong significance ($P < 0.01$) in association with the MDS populations variability (**Figure 2A**).The trait correlation within the MDS space revealed trait association and trade-offs as the case of seed morphology and size parameters that appeared in the opposite direction of seed production traits (**Figure 2A**). Regarding the morphometric analysis, post-hoc tests revealed that even close populations have different seed mass (**Figure 2B**). The smallest seeds were found in M1 with an average seed mass of 19.44±0.82 µg and a basal seed area of 0.81±0.04 mm^2 whereas the heaviest seeds were found in CB2 with 28.04±0.01 µg, and a basal area of 1.00±0.02 mm^2 (**Figure 2, Table S1**). Besides seed morphology, the near *C. edulis* populations also differed in seed production, viability, germination, biochemistry and persistence **(Figures 2**). Total seeds per fruit ranged from 57.73±42.80 in CB3 to 2,478.45±345.32 seeds in CB1. In spite of the huge standard error those differences resulted significant (**Figure 2C**), showing a more than 40 times higher average seed production at less than 1 km of distance. Seed viability ranged from 71 to 88% but germination ranged from 22% to 80% across populations, arising differences in the percentage of dormant seeds (**Figure 2D**). The most abundant tocochromanol found, γ-tocopherol, also differed significantly between near populations (**Figure 2F**). Seeds γ-tocopherol concentration ranged from 5.43±0.44 in CC2 to 7.55±0.30 mg gDW^{-1} in CB1 (**Figure 2F**), whereas no significant differences were found in its successor, α-tocopherol, that remained around 0.27±0.01 mg gDW^{-1} (**Table 1, S1**). Longevity analysis revealed differences between zones but only M2 showed decreased seed mean longevity in terms of L_{50} (**Figure 2G**).

Considering the three geographically distant zones, seeds from the populations at Costa Brava (CB) showed significantly higher viability percentages and higher longevity compared to Maresme and Cap de Creus but high variability was found between populations within each zone (**Figure 2, 2**). Significantly lower germination percentages were found in Cap de Creus seeds although big variability was found between populations, from 25% to 80% of germinated seeds (**Figure 2**). Costa Brava seeds showed significantly lower mean germination time (about two weeks), whereas mean germination time for seeds from other populations raised to 30 days (**Table S1**). The highest percentage of dormancy was found in a Costa Brava population in spite of the high variability found within populations, from 20 to 60% of dormant seeds (**Table S1**).

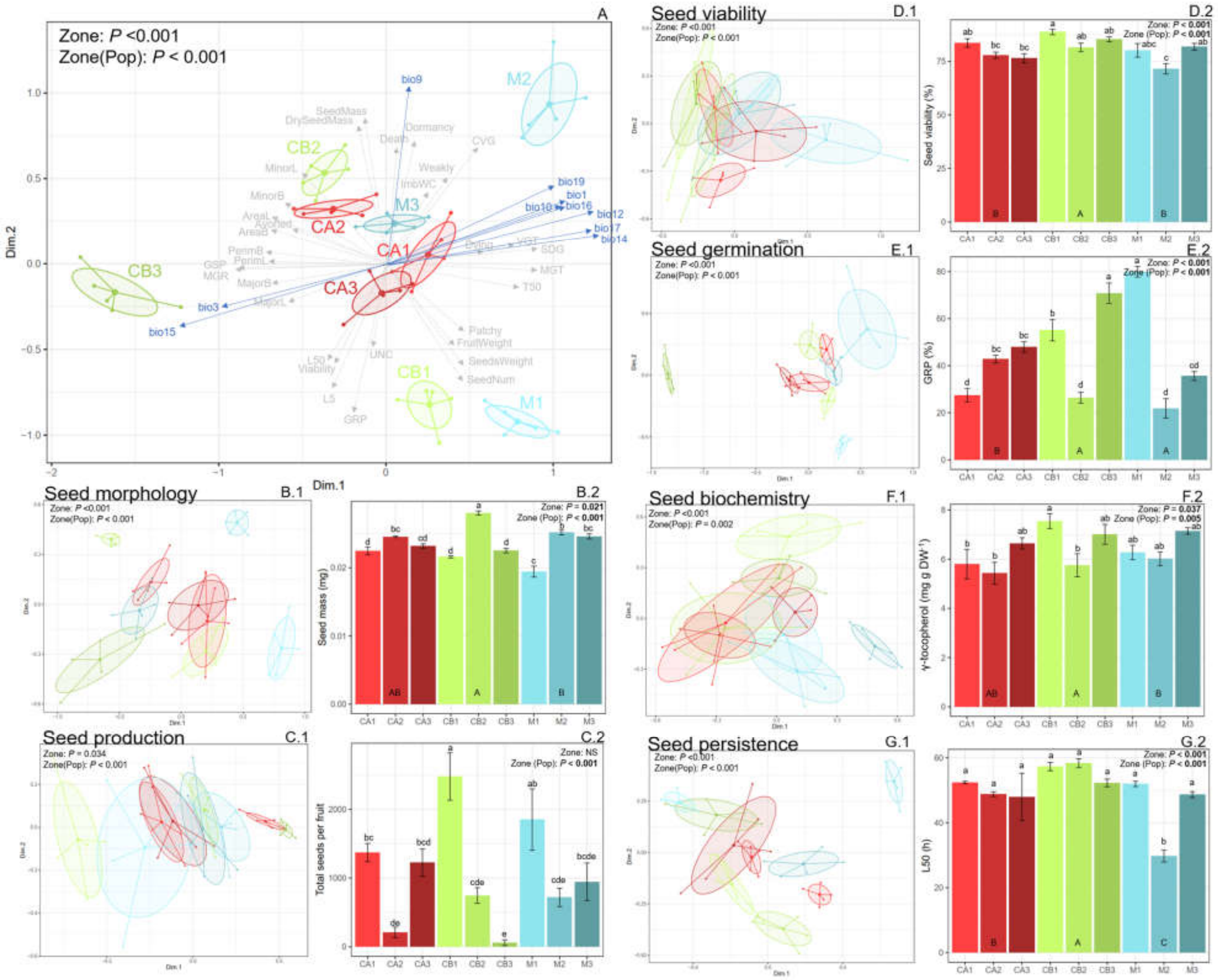


Figure 2. **A**. MDS for all measured traits including variables that significantly strongly ($P < 0.01$) contribute to population variability among measured traits (grey) and bioclimatic external variables (blue). Ellipses represent 95% of confidence intervals. *P*-values correspond to PERMANOVA results. **B.1, C.1, D.1, E.1, F.1, G.1** show seed morphology, production, viability, germination, biochemistry and persistence MDS respectively and **B.2, C.2, D.2, E.2, F.2, G.2** relevant seed trait from each trait group respectively. Complete traits list and population means for all traits can be found in Table 1 and Table S1 respectively. Different capital letters show differences between zones, whereas different small letters show significant differences between populations. Data is shown as Mean ± SE, N = 5.

Strong spatial variation in seed production, viability, germination and persistence

Correlation of geographic and environmental distances with trait's distances across populations did not result in any strong correlation in most of the studied traits, as revealed by the Mantel tests (**Table 1**). Only populations distance of the seed water content after 24h of imbibition (WC_Imb) and the percentage of dying seeds (Dying) showed a correlation coefficient superior to 0.5 with geographic distance ($P = 0.001$, $R_M = 0.538$) (**Table 1**), this is, the distant the population, the different imbibed water content. Poor correlation coefficients for the Mantel test were also obtained when contrasting environmental and geographic distance with the multivariate scaled seed traits distance between populations (Table 1). When traits variability was translated into percentual trait change with the geographic distance, different patterns considering the different traits type were observed, with impressive variation at small geographic scales (**Figure 3**). In concordance with the weak regressions found in populations seed traits and geographic distance in the Mantel test, no clear trend is observed in percentual trait variability with increasing geographic distance. However, it is noticeable that the percentual variability scale shows strong variation among group traits. The strongest percentual trait variability was found in seed production traits (up to 500% mean values change with distance) whereas the smallest percentual trait variability was found in seed morphology traits (up to 20% mean values). Seed viability, germination and persistence showed also a disparate variation from 0 to 250%. The measured seed biochemical traits showed small percentual variation with geographic distance (under 20%), with the exception of the imbibed seed water content that was indeed the only strong correlation in the Mantel test (**Table 1**) and it increased from 15 to 50% of percentual variation with distance (**Figure 3**).

Not just the mean percentual variation but the percentual variability at the closer scale is surprisingly high, revealing that populations at less than 1 km may potentially have a 700% different seed production, 300% different seed viability and persistence and 200% different seed germination, but just a 20% different seed morphology and biochemistry (**Figure 3**).

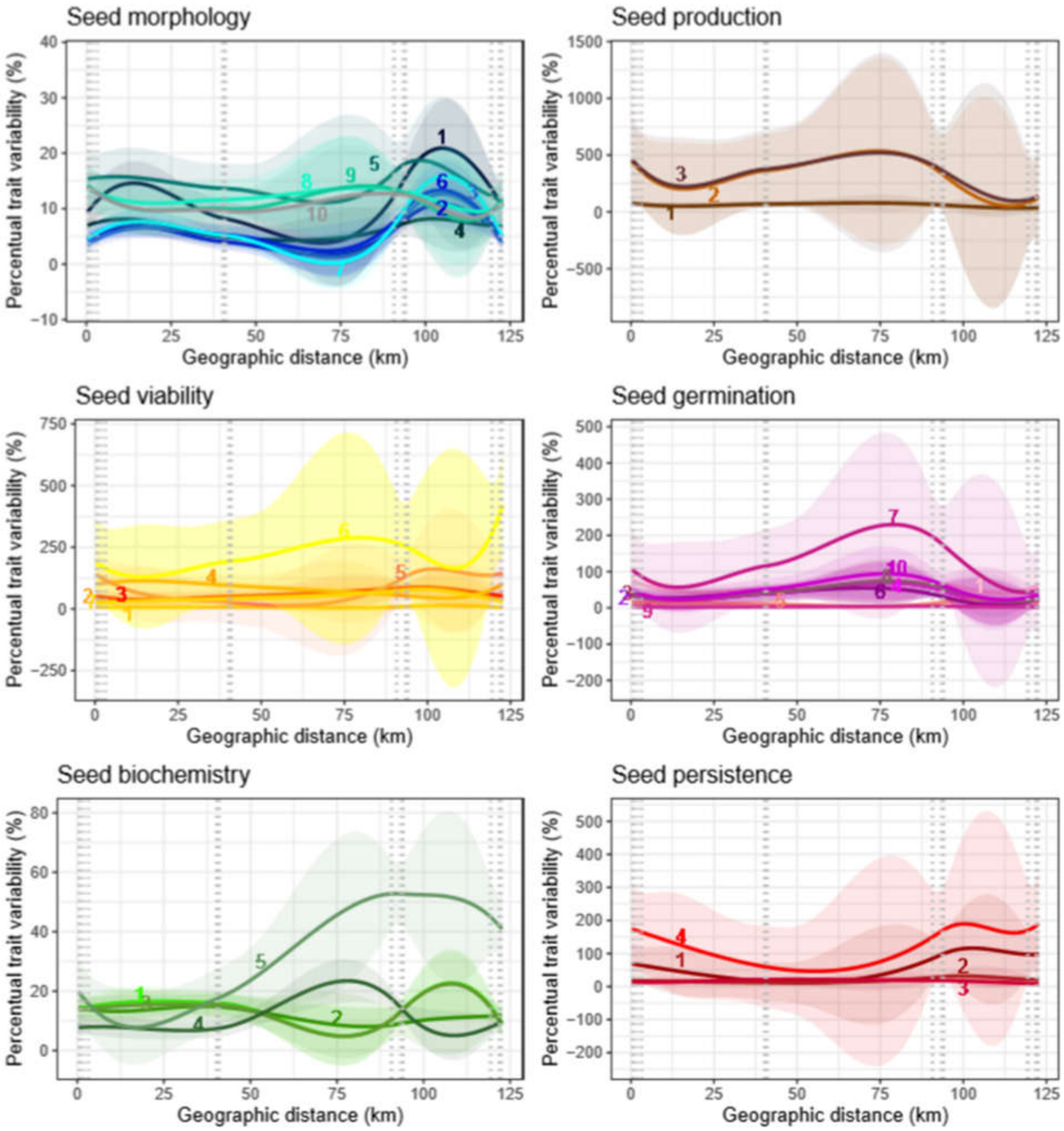


Figure 3. Geographic percentual change in the different measured traits regarding seed morphology, production, viability, germination, biochemistry and persistence. Dotted lines constitute measured locations. Numbers correspond to seed traits order per trait type in Table 1. Shaded areas define the LOESS model standard error for each variable.

Zone and climate influence trait spatial variability

Despite the high geographic variability found in almost all seed traits, this was not homogeneous among zones. The variability found between the near populations in the area of Cap de Creus was significantly lower than the variation between near populations in the other zones (**Figure 4A**). However, this smaller variation in the Cap de Creus area was not constant among the 39 traits (**Figure 4B**). Smaller variation in Cap de Creus was detected for: seed mass, dry seed mass, imbibed seed water content seed number, GRP, CVG and T_{50}. In general, differences in traits variability were detected for most seed viability, persistence and germination traits (**Figure 4B**).

Seed traits variability was contrasted with the 19 bioclimatic variables from the WorldClim model and revealed strong and significant correlations with most traits (**Figure 4C**). The bioclimatic variables with

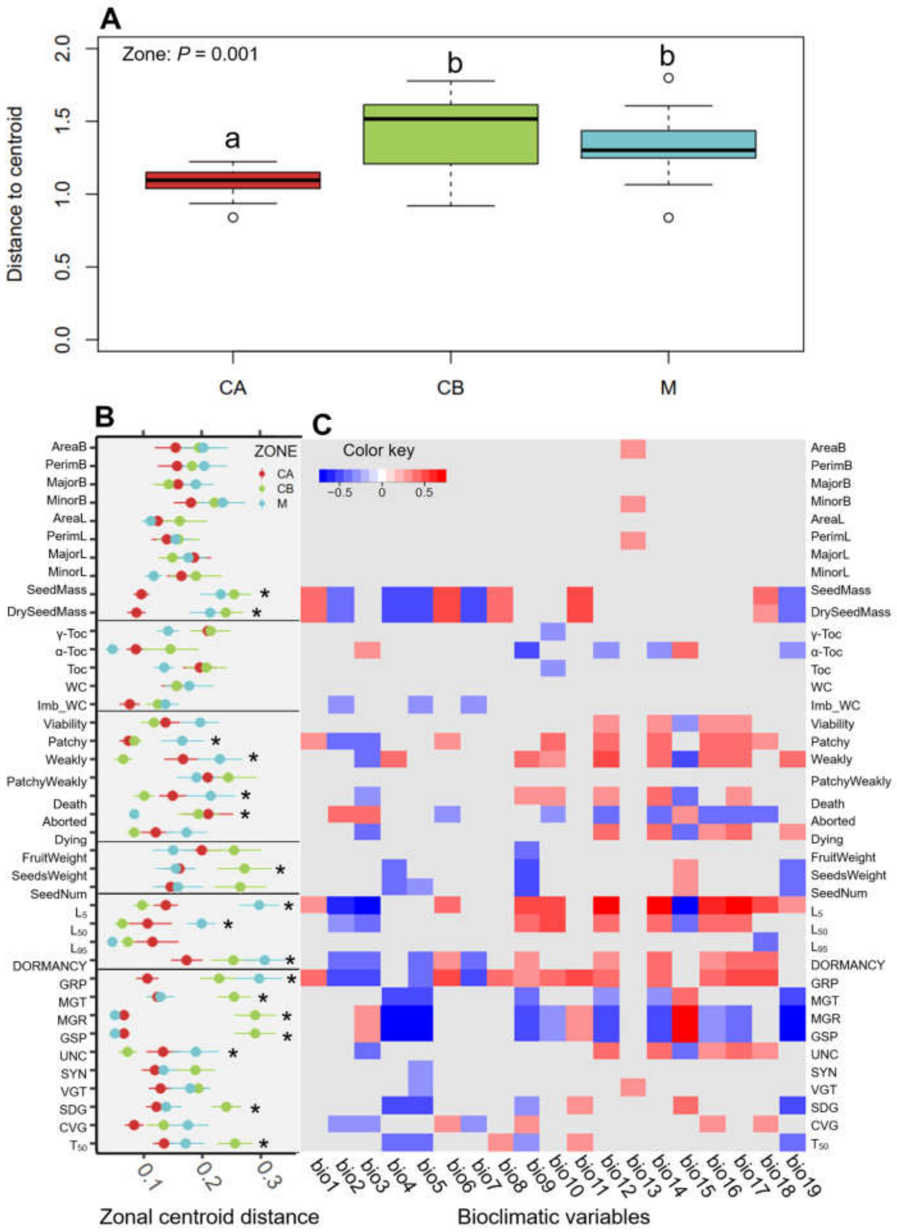


Figure 4. Seed traits variability and the influence of the environment. **A.** Distance to zonal centroid comparison using all traits. Different letters show significant differences between zones. **B:** Zonal centroid distance for each trait in each zone. Asterisks (*) represent significant differences between zones. Data is shown as Mean ± SE. **C:** Bioclimatic variables Pearson correlations with zonal centroid distance for all measured seed traits. Coloured correlations are significant and blue to red palette represent the R coefficient. Complete bioclimatic variables names can be found in http://www.worldclim.org/bioclim.

strongest significative correlations ($P < 0.05$, $R^2 > 0.6$) with traits variability were: bio3 (isothermality), bio4 (temperature seasonality), bio5 (Max. temperature of the warmest month), bio12 (annual precipitation), bio15 (precipitation seasonality), bio16 (precipitation of the wettest quarter), bio17 (precipitation of the driest quarter) and bio19 (precipitation of the coldest quarter). Most of those bioclimatic variables also correlated significantly with traits variation across populations ($P < 0.01$, **Figure 2A**). Among those variables, some showed a clear negative correlation with most traits' variability. Decreased isothermality, temperature seasonality, the maximum temperature of the warmest month and the precipitation of the coldest quarter correlated with increased zonal traits variability. In the other hand, annual precipitation, precipitation of the driest month, precipitation seasonality, precipitation of the wettest quarter and precipitation of the driest quarter showed both strong negative and positive correlations. The strongest correlation found was a negative correlation between the precipitation of the coldest quarter and mean germination rate (MGR) variability ($P < 0.05$, $R^2 = -0.752$). Besides MGR, L_5 showed also strong significant ($P < 0.05$) correlations with bioclimatic variables: isothermality and precipitation seasonality correlated with decreased L_5 variability, whereas annual precipitation, precipitation of driest month, wettest quarter and driest quarter correlated with increased L_5 variability. The higher L_5 variability is found in sites with higher precipitation among the year and variable temperatures.

Discussion

Variability in seed traits in geographically near populations

As novel ecosystem players, invasive species are highly affected by heterogeneous environmental pressures across the landscape and therefore high intraspecific variability may be expected, especially regarding plant seed traits that compromise species persistence and expansion. The obtained results revealed the existence of high seed traits intraspecific variability or functional β-diversity considering different populations of an invasive species, regardless environmental and geographic distance. *C. edulis* populations located at less than 1 km of distance, showed differential seed production, seed mass, seed germination percentage, mean germination time, seed longevity and dormancy percentages and these differences between populations may suppose differential soil seed bank dynamics at the different sites. The population of Carrer del Golf (CB2) for example, showed a similar seed trait's profile to Far de s'Arenella (CC1) and Can Teixidor (M2), located at 40 and almost 100 km of distance respectively. Those three populations have high proportions of dormant viable seeds (**Figures 2, 3**). Moreover, the ca. 1,000 seeds produced per fruit in those populations contribute to forecast the formation of a soil seed bank at the described locations. Contrarily, the population Puig Sa Guilla (CB3) that showed a small seed production (less than 100 seeds per fruit), have the highest germination percentages and the lowest germination time, revealing low dormancy ratios, similarly to the population at Ca l'Antic (M1). Variability in achene traits was assessed in *Fallopia x bohemica* in different French populations, revealing differential dispersion strategies between populations, which may facilitate the colonization of contrasting environments (Lamberti-Raverot *et al*., 2019). Similarly, the seed traits plasticity in *C. edulis* determines plastic strategies regarding seed bank dynamics. Some of the analysed *C. edulis* populations showed a persistence strategy with the formation of a soil seed bank whereas others showed a more expansive strategy (high germination rates). The high viability rates in the invasive populations suggest a high sexual reproduction vigour complementing the high asexual capacity of this clonal species (Roiloa, 2019).

Seed traits types have different variation magnitudes. Seed traits regarding seed production and seed viability were the most variable across near populations of *C. edulis*. In the study developed by Bogdziewicz *et al*. (2019) with masting species (species with synchronized and highly variable levels of seed production over years within a population or a community) seed production was found to be not correlated with geographic distance in *Quercus petrea* but correlated in *Fagus sylvatica*. The authors suggest that this difference is associated with the species masting type and their sensitivity to environmental conditions. *F. sylvatica* is a flowering masting species (variability is driven by variable flowering effort) whereas *Q. petrea* is a fruit-maturation masting species (variation in fruit production is driven by the variable ripening of a more constant flower crop). Due to the large period of fruit maturation and desiccation in *C. edulis* (from May to January in the Mediterranean basin), it is hypothesized that the observed variability in seed production in *C.*

edulis may be similar to a fruit-maturation masting species with geographic distance independence as *Quercus petrea*. Mother plant water stress has been described to compromise seed viability and dormancy in *Astragalus nitidiflorus* growing at the Mediterranean area (Segura *et al*., 2015), and may be the cause of *C. edulis* observed variability in seed viability traits among near populations. In accordance with this, significant correlations were found between variability in seed production traits and bioclimatic variables suggesting higher seed production variability with lower temperature seasonality, mean temperature of the driest quarter, precipitation of the coldest quarter and higher precipitation seasonality.

Invasive seeds variability role on the invasion process and management implications

In the present study the high variability found in seed traits between close and distant populations of *C. edulis* lead to differentiated strategies at the different populations regardless their geographic or environmental distance, suggesting a high degree of local adaptation. Strong evolutionary pressures interact when an exotic species is introduced into a new habitat that may lead to rapid adaptation processes. During *Lythrum salicaria* expansion in North America, rapid local adaptation in terms of earlier flowering played a crucial role in the success of this invasive species (Colautti and Barrett, 2013). This local adaptation is described to be independent of the geographic distance of nearest populations (Leimu and Fischer, 2008). High variability in seed traits of the invasive species *Fallopia x bohemica* has been described across different populations, revealing differential dispersion strategies that may favour adaption and colonisation of different habitats (Lamberti-Raverot et al., 2019). *F. bohemica* plasticity in lamina size, growth and biomass allocation may facilitate the colonization of contrasting environments (Walls, 2010). In *C. edulis*, highly plastic responses have been described in terms of photoprotective strategies (Fenollosa et al., 2017), biomass allocation and morphological variations that contribute to its invasive success (Traveset, Moragues and Valladares, 2008; Roiloa et al. 2014; Roiloa et al. 2016). The results of *C. edulis* seed traits variability within and between close populations that share similar environmental conditions also suggest high plasticity in seed traits that may contribute to species success allowing local adaptation.

Not just plastic responses but also genetic effects may determine intraspecific traits variability (Albert et al., 2011). In clonal species, environment-dependent heritable epigenetic changes may contribute to adaptive genotypes (Verhoeven and Preite, 2014). Epigenetic transmission in *C. edulis* has been proposed to be particularly important due the rapid generation turnover through its grow and die strategy (Fenollosa et al., 2016). Moreover, *C. edulis* shows slight agamospermy (i.e. asexual seed production) (Vilà, *et al*., 1998), which may facilitate the fixation of genetic combinations over multiple generations and promote rapid local adaptation as shown by the study conducted with different *Cenchrus* species that concluded that agamospermy facilitates the expansion of species by enabling reproduction even under stressful conditions (Kumar *et al*., 2019). Both mechanisms, epigenetic changes and agamospermy may contribute to genotypic fixation in *C. edulis* and contribute to trait variability in addition to plasticity. A recent study with the clonal invader *Plantago lanceolata* revealed that in the invasive range, repeated, long-distance, human-mediated introductions promoted high genetic diversity but the lack of spatial genetic structure due to admixture in comparison with the native regions that showed a strong genetic structure (Smith et al., 2020). The absence of a correlation between traits variability and geographic distance among the analysed populations and zones of *C. edulis* does not support the hypothesis of the existence of an expansion gradient or functional structures for example from urban to natural areas. Instead, the obtained results reveal that there are no identical populations in terms of seed traits as the nearest populations can be the less similar in terms of multidimensional seed traits distance.

The found variability in seed traits between *C. edulis* populations may have important implications for this invasive species management. The work developed by Loddo *et al*. (2018) that evaluated intraspecific variability in the invasive species *Abutilon theophrasti* seed traits demonstrated that achieving a better knowledge of interpopulation variability can allow specific control strategies to be designed, facilitating the development of new management tools. Likewise, germination rates of the Alexandra palm were found to potentially better inform of management strategies for the control of this species (Wen, 2019). Considering the obtained results in *C. edulis*, on one hand the differential seed behaviour may determine differential requirements in soil management after eradication. Populations such as CB2, CC1 and M2, with high seed dormancy rates may require management cost at the long term after eradication as recruitment may be expected from the soil seed bank. On the other hand, the ability to form persistent seed banks might contribute substantially to determine the invasion potential of alien plants in their new distribution ranges, given the role of seed banks as sources of propagules, genetic diversity, and in spreading the risk of germination failure over time (Gioria *et al*., 2019). Therefore, the inclusion of soil seed bank dynamics analysis during prioritization of eradication zones may help to develop cost-effective management strategies, by increasing investment in post-eradication management in populations with high seed longevity and prioritizing populations with strong seed production. Moreover, other seed traits may be also considered when prioritizing eradication zones as have been associated high higher invasive character. For example, lower seed mass helped to explain invasive success in Hawaii (Schmidt and Drake, 2011), and higher seed production is associated with increased invasive potential (Pyšek and Richardson, 2007).

Determinants of seed traits variability: environment and disturbance

When unravelling the determinants of intraspecific seed trait variability in *C. edulis*, both environment and disturbance seem to play a major role, whereas geographic distance may not. The particular environmental variables that the species encounter in each population may contribute to seed traits spatial variability, as most of the analysed seed traits correlated with bioclimatic variables. However, traits distance did not correlate with environmental distance between populations, suggesting that in spite of the fact that environmental variables influence seed traits, they did not generate a gradient of different seed phenotypes. In *Fagus sylvatica* seed production correlated with mean temperature of the warm period but also variability increased with hot (Bogdziewicz *et al*., 2019). Higher dispersal distance with increasing temperatures has been described for the invasive species *Carduus nutans* (Zhang *et al*., 2011). The high variability in the expression of the seed traits assessed among invasive Chilean *Taraxacum officinale* populations appeared to be highly related to experienced variation in rainfall (Molina-Montenegro *et al*., 2018) revealing the importance of environmental conditions or seasonality in determining seed traits variability. Recently, the study of global gradients for intraspecific trait variation with almost 3,000 species revealed pronounced associations with climate, with lower variation values in colder areas and higher values in drier areas (Kuppler et al., 2020). In concordance with this recent study, seed traits variation in *C. edulis* was generally higher in zones with lower precipitation of the coldest quarter (bio19) but also with maximum temperature of the warmest month (bio5) arising that in this case we found increased variability in colder and drier areas that may induce stressful conditions for this species. In accordance with this, a previous study of *C. edulis* physiological response to cold revealed that introduced individuals have an increased chilling tolerance in comparison to native individuals, suggesting that low temperatures constitute a major abiotic pressure in the introduced range (Fenollosa and Munné-Bosch, 2019). The obtained results in *C. edulis* are relevant for evaluating climate change impacts as for example in Australia, a study demonstrated that the environmental conditions expected under climate change scenarios may potentially cause seed longevity to decline for a species in arid climates, impacting on the native community composition (Kochanek *et al*., 2010). Zones with lower maximum temperatures during the warmest month have higher seed traits variability in *C. edulis*, suggesting that global warming may have an impact on the invasion of this species decreasing seed variability. However, changes towards reduced pluviometry expected in a global change framework (Christensen et al., 2017) may exhort contrary effects and thus further research must be addressed to assess global change effects on *C. edulis* seed traits variability. In general, the review conducted by Johnson *et al*., (2019) revealed that several seed dispersion parameters are expected to be altered with climate change, revealing complex ecosystem assemblance in the near future.

Besides the environmental effect, the magnitude of the variability in *C. edulis* seed traits found between the different close populations was different between the three contrasted zone with human presence gradient. The area with lowest human presence, the zone of Cap de Creus, was the zone where smallest variation in *C. edulis* seed traits was registered. Urbanization is land-use change form characterized by increased human impacts, fragmentation of natural habitats, intensive land transformation and an increase in impervious surface land cover thus reducing the spatial extent for vegetation

(Irwin and Bockstael 2007; Williams et al., 2015). Urban environments create a wide range of novel habitats such as industrial areas or waste dumps that may constrain species performance and community assemblance (Irwin and Bockstael 2007). This increased variability in novel habitats associated with urban areas may be responsible for the increased seed traits variability found in *C. edulis* zones with intense anthropogenic pressure. In this way, the meta-analysis conducted by Williams et al. (2015) already exposed that the observed variability in urban plant traits, including seed traits, is linked to the consistency and strength of urban stressors. This result, in concordance with the framework presented by Gaertner et al., (2017), suggest that anthropogenic pressure in urban areas may exacerbate the invasive species impacts not only by acting as launching sites but also by increasing its intraspecific variability of species within urban environments. In the case of *C. edulis*, the study of Lechuga-Lago et al., (2017) revealed that there is a strong link between invasion and urban areas, as urbanisation assist *C. edulis* invasion which also increase the impact of urbanisation.

Our study describes high variability on the seed traits of *C. edulis* between near (<4 km) and distant populations (> 40km), revealing a high functional β-diversity for this species, which is not explained by geographic distance. The observed variability in the *C. edulis* seed traits towards high germination rates in some populations versus high dormancy rates in others reveal differential soil seed bank dynamics that may require differential strategies for a cost-effective management. Seed traits intraspecific variability was found to be influenced by environmental conditions suggesting a potential impact of climate shifts. Moreover, the increasing human presence gradient between zones suggest that high anthropogenic pressure may increase seed traits variability which may contribute to exacerbate the invasive species impacts in urban areas.

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Literature cited

Affre L. 2011. Ecological and evolutionary insights from plant invasions: the case of *Carpobrotus spp*. (Aizoaceae) in the Mediterranean Basin. In: Berhardt LV, eds. *Advances in Medicine and Biology*. New York: Nova Science Publishers.

Albert CH, Grassein F, Schurr FM, Vieilledent G, Violle C. 2011. When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics* 13: 217–225.Amaral JS, Casal S, Torres D, Seabra RM, Oliveira BPP. 2005. Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method. *Analytical Sciences* 21: 1545–1548.

Bailly C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Science Research* 14: 93–107.

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H. 2013. *Seeds: Physiology of Development, Germination and Dormancy*, 3rd Edition, Springer, New York.

Bogdziewicz M, Szymkowiak J, Fernández-Martínez M, Peñuelas J, Espelta JM. 2019. The effects of local climate on the correlation between weather and seed production differ in two species with contrasting masting habit. *Agricultural and Forest Meteorology* 268: 109-115.

Carrillo E, Ferré A, Illa E, Mercadé A, eds. 2018. *Mapa de vegetació de Catalunya, E 1:50.000*. Barcelona: Universitat de Barelona.

Chenot J, Affre L, Passetti A, Buisson E. 2014. Consequences of iceplant (*Carpobrotus*) invasion on the vegetation and seed bank structure on a Mediterranean island: response elements for their local eradication, *Acta Botanica Gallica* 161:3 301-308.

Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held R, et al. 2007. Regional climate projections. In Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. *Climate Change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge: Cambridge University Press.

Colautti RI, Barrett SCH. 2013. Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science* 18: 364-366.Fenollosa E, Roach DA, Munné-Bosch S. 2016. Death and plasticity in clones influence invasion success. *Trends in Plant Science* 21(7): 551–553.

Fenollosa E, Munné-Bosch S, Pintó-Marijuan M. 2017. Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*. *Physiologia Plantarum* 160: 185–200.

Fenollosa E, Munné-Bosch S. 2019. Increased chilling tolerance of the invasive species *Carpobrotus edulis* may explain its expansion across new territories. *Conservation Physiology* 7:1 1-15.

Fenollosa E, Jené L, Munné-Bosch S. 2020. A rapid and sensitive method to assess seed longevity through accelerated aging in an invasive plant species. *Plant Methods*. https://doi.org/10.1186/s13007-020-00607-3

Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37: 4302–4315.

Gaertner M, Wilson JRU, Cadotte MW, MacIvor JS, Zenni RD, Richardson DM. 2017. Non-native species in urban environments: patterns, processes, impacts and challenges. *Biological invasions* 19: 3461-3469.

Gioria M, Le Roux JJ, Hirsch H *et al*. 2019. Characteristics of the soil seed bank of invasive and non-invasive plants in their native and alien distribution range. *Biological Invasions* 21: 2313–2332.

Gioria M, Pyšek P, Osborne BA. 2018. Timing is everything: does early and late germination favor invasions by herbaceous alien plants? *Journal of Plant Ecology* 11:1 4–16.

Gioria M, Pyšek P. 2016. The legacy of plant invasions: changes in the soil seed bank of invaded plant communities. *BioScience* 66:1 40–53.

Gioria M, Pyšek P. 2017. Early bird catches the worm: germination as a critical step in plant invasion. *Biological Invasions* 19: 1055.

Guido A, Hoss D, Pillar VD. 2017. Exploring seed to seed effects for understanding invasive species success. *Perspectives in Ecology and Conservation* 15:3 234-238.

Hay FR, Valdez R, Lee J-S, Sta. Cruz PC. 2019. Seed longevity phenotyping: recommendations on research methodology. *Journal of Experimental Botany* 70: 425–434.

Huang F, Peng S, Chen B, Liao H, Huang Q, Lin Z, Liu G. 2015. Rapid evolution of dispersal-related traits during range expansion of an invasive vine *Mikania micrantha*. *Oikos* 124: 1023-1030.

Irwin EG, Bockstael NE. 2007. The evolution of urban sprawl: Evidence of spatial heterogeneity and increasing land fragmentation. *Proceedings of the National Academy of Sciences of the United States of America* 104: 20672–7.

Januchowski-Hartley SR et al. 2018. The need for spatially explicit quantification of benefits in invasive-species management. *Conservation Biology* 32: 287–293

Jarić I, Heger T, Castro Monzon F, Jeschke JM, Kowarik I, McConkey KR, Pyšek P, Sagouis A, Essl F. 2019. Crypticity in Biological Invasions. *Trends in Ecology & Evolution* 34:4 291-302.

Johnson JS, Cantrell RS, Cosner C, Hartig F, Hastings A, Rogers HS, Schupp EW, Shea K, Teller BJ, Yu X, Zurell D, Pufal G. 2019. Rapid changes in seed dispersal traits may modify plant responses to global change. *AoB PLANTS* 11:3, plz020.

Jucker T, Carboni M, Acosta AT. 2013. Going beyond taxonomic diversity: deconstructing biodiversity patterns reveals the true cost of iceplant invasion. *Diversity and Distribution* 19:1566–1577.Kochanek J, Buckley YM, Probert RJ, Adkins SW, Steadman KJ. 2010. Pre-zygotic parental environment modulates seed longevity. *Austral Ecology* 35: 837-848.

Kucera B, Cohn M, Leubner-Metzger G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Science Research* 15:4 281-307.

Kumar S, Saxena S, Rai A, Radhakrishna A, Kaushal P. 2019. Ecological, genetic, and reproductive features of *Cenchrus* species indicate evolutionary superiority of apomixis under environmental stresses. *Ecological Indicators* 105: 126-136.

Kuppler J, Albert CH, Ames GM, et al. 2020 Global gradients in intraspecific variation in vegetative and floral traits are partially associated with climate and species richness. *Global Ecology and Biogeography:* 00: 1–16.Labouriau LG, Valadares MEB. 1983. *The germination of seeds.* OEA, Washington, DC.

Lamberti-Raverot B, Piola F, Vallier F, Gardette V, Puijalon S. 2019. Achene traits involved in the water dispersal of the invasive *Fallopia* × *bohemica* complex: Variability and dispersal strategies. *Flora* 251: 88-94.

Lechuga-Lago Y, Novoa A, Le Roux JJ, Gonzalez L. 2017. Understanding the influence of urbanization on invasibility: *Carpobrotus edulis* as an exemplar. *Biological Invasions* 19: 3601–3611.Leimu R, Fischer M. 2008. A meta-analysis of local adaptation in plants. *PLOS ONE* 3:12 e4010.

Loddo D, Bozic D, Calha IM, Dorado J, Izquierdo J, Šćepanović M, Barić K, Carlesi S, Leskovsek R, Peterson D, Vasileiadis VP, Veres A, Vrbničanin S, Masin R. 2019. Variability in seedling emergence for European and North American populations of Abutilon theophrasti. *Weed Research* 59: 15– 27.

Mitchell J, Johnston IG, Bassel GW. 2017. Variability in seeds: biological, ecological, and agricultural implications. *Journal of Experimental Botany* 68:4 809–817.

Molina-Montenegro MA, Acuña-Rodríguez IS, Flores TSM, Hereme R, Lafon A, Atala C, Torres-Díaz C. 2018. Is the success of plant invasions the result of rapid adaptive evolution in seed traits? Evidence from a latitudinal rainfall gradient. *Frontiers in Plant Science* 9:208.

Molinari N, D'Antonio C, Thomson G. 2007. Carpobrotus as a case study of the complexities of species impacts. In: Cuddington K, Byers JE, Wilson WG, Hastings A, eds. *Ecosystem Engineers: Plants to Protists.* London: Elsevier, 139–162.

Novoa A, González L, Moravcová L, Pyšek P. 2013. Constraints to native plant species establishment in coastal dune communities invaded by *Carpobrotus edulis*: Implications for restoration. *Biological Conservation* 164: 1–9.

Novoa A, González L. 2014. Impacts of *Carpobrotus edulis* (L.) NE Br. on the germination, establishment and survival of native plants: a clue for assessing its competitive strength. *PLoS One* 9: e107557.

Passos I, Marchante H, Pinho R. et al. 2017. What we don't seed: the role of long-lived seed banks as hidden legacies of invasive plants. *Plant Ecology* 218: 1313–1324.Preston CD, Sell PD. 1988. The Aizoaceae naturalized in the British Isles. *Watsonia* 17 (3): 217–245.

Pyšek P, Richardson DM. 2007. Traits associated with invasiveness in Alien plants: where do we stand? In: Nentwig W (ed) *Biological invasions*, vol 193. Ecol Stud. Springer Verlag, BerlinRanal MA, Santana DG. 2006. How and why to measure the germination process? *Revista Brasileira de Botanica* 29:1 1-11.

Roiloa S. 2019. Clonal traits and plant invasiveness: The case of Carpobrotus N.E.Br. (Aizoaceae). *Perspectives in Plant Ecology, Evolution and Systematics* 40: 125479.

Roiloa SR, Retuerto R, Campoy JG, Novoa A, Barreiro R. 2016. Division of labor brings greater benefits to clones of *Carpobrotus edulis* in the non-native range: evidence for rapid adaptive evolution. *Frontiers in Plant Science* 7:394 1–13.

Roiloa SR, Rodriguez-Echeverría S, López-Otero A, Retuerto R, Freitas H. 2014. Adaptive plasticity to heterogeneous environments increases capacity for division of labor in the clonal invader *Carpobrotus edulis* (Aizoaceae). *American Journal of Botany* 101: 1301–1308.

Ruffino L, Krebs E, Passetti A, Aboucaya A, Affre L, Fourcy D, Lorvelec O, Barcelo A, Berville L, Bigeard N, Brousset L, De Méringo H, Gillet P, Le Quilliec P, Limouzin Y, Médail F, Meunier JY, Pascal M, Ponel P, Rifflet F, Santelli C, Buisson E, Vidal E. 2015. Eradications as scientific experiments: progress in simultaneous eradications of two major invasive taxa from a Mediterranean island. *Pest Management. Sciences* 71: 189-198.

Saatkamp A, Cochrane A, Commander L, Guja L, Jimenez-Alfaro B, Larson J, Nicotra A, Poschlod P, Silveira FAO, Cross A, Dalziell EL, Dickie J, Erickson TE, Fidelis A, Fuchs A, Golos PJ, Hope M, Lewandrowski W, Merritt DJ, Miller BP, Miller R, Offord CA, Ooi MKJ, Satyanti A, Sommerville KD, Tangney R, Tomlinson S, Turner S, Walck JL. 2019. A research agenda for seed-trait functional ecology. *New Phytologist* 221: 1764-1775.

Sallon S, Solowey E, Cohen Y, Korchinsky R, Egli M, Woodhatch I, Simchoni O, Kislev M. 2008. Germination, genetics, and growth of an ancient date seed. *Science* 320:5882 1464.

Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M, DellaPenna D. 2004. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *Plant Cell* 16: 1419–1432.

Schmidt JP, Drake JM. 2011. Time since introduction, seed mass, and genome size predict successful invaders among the cultivated vascular plants of Hawaii. *PLoS ONE* 6: e17391.

Segura F, Vicente MJ, Franco JA, Martínez-Sánchez JJ. 2015. Effects of maternal environmental factors on physical dormancy of *Astragalus nitidiflorus* seeds (Fabaceae), a critically endangered species of SE Spain. *Flora - Morphology, Distribution, Functional Ecology of Plants* 216: 71-76.

Traveset A, Moragues E, Valladares F. 2008. Spreading of the invasive *Carpobrotus* spp. in Mediterranean ecosystems: the advantage of performing well in different light environments. *Applied Vegetation Science* 11:45–54.

Verhoeven KJ Preite V. 2014. Epigenetic variation in asexually reproducing organisms. *Evolution* 68: 644-655.

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron, JL, Pergl J, Schaffner U, Sun Y, Pyšek P. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14: 702-708.

Vilà M, Valladares F, Traveset A, Santamaría L, Castro P,eds. 2008. *Invasiones Biológicas.* CSIC, Madrid: Spain.Walls RL. 2010. Hybridization and plasticity contribute to divergence among coastal and wetland populations of invasive hybrid Japanese knotweed sl (*Fallopia* spp.). *Estuaries Coasts* 33: 902–918.

Walters C, Wheeler LM, Grotenhuis JM. 2005. Longevity of seeds stored in gene bank: species characteristics. *Seed Science Research.* 15: 1–20.

Wen B. 2019. Seed germination ecology of Alexandra palm (*Archontophoenix alexandrae*) and its implication on invasiveness. *Scientific Reports* 9: 4057.

Williams NSG, Hahs AK, Vesk PA. 2015. Urbanisation, Plant Traits and the Composition of Urban Floras. *Perspectives in Plant Ecology, Evolution and Systematics* 17: 78–86.

Zhang R, Jongejans E, Shea K. 2011. Warming increases the spread of an invasive thistle. *PLoS One* 6: e21725

Tables

Table 1. List of measured traits with their acronyms, ecological relevance, potential for invasiveness and Mantel Correlation coefficient (R_M) and *P*-values for the Mantel test contrasting the distance between populations of each trait or trait group versus the geographic and environmental distances. NS = Not significant

					Mantel Test			
Trait Type	**Trait**	**Acronym**	**Ecological relevance**	**Potential for invasiveness**	**Geographic distance**		**Environmental distance**	
Morphometry	Basal seed area (mm^2)	AreaB	Descriptors of seed size and shape, directly related to dispersal as they influence abiotic and biotic travel distance, determining how and how efficiently seeds disperse.	Seed morphological traits may determine differential dispersion strategies that can favor adaption and colonization of different novel habitats.	*P* = 0.048, RM = 0.077	P = 0.018, RM = 0.108	*P* = 0.025, RM = 0.068	*P* = 0.015, RM = 0.089
Morphometry	Basal seed perimeter (mm)	PerimB			*P* = 0.031, RM = 0.104		*P* = 0.027, RM = 0.071	
Morphometry	Major basal diagonal (mm)	MajorB			*P* = 0.021, RM = 0.104		*P* = 0.005, RM = 0.111	
Morphometry	Minor basal diagonal (mm)	MinorB			NS		NS	
Morphometry	Lateral seed area (mm^2)	AreaL			NS		NS	
Morphometry	Lateral seed perimeter (mm)	PerimL			*P* = 0.046, RM = 0.071		*P* = 0.047, RM = 0.065	
Morphometry	Major lateral diagonal (mm)	MajorL			*P* = 0.045, RM = 0.076		*P* = 0.033, RM = 0.076	
Morphometry	Minor lateral diagonal (mm)	MinorL			NS		NS	
Morphometry	Seed mass (mg)	SeedMass			NS		NS	
Morphometry	Dry seed mass (mg)	DrySeedMass			NS		NS	
Biochemistry	Gamma tocopherol (mg/gDW)	γ-Toc	Tocopherol seed content has a critical role in maintaining seed viability and protecting lipids from oxidation during germination and early seedling growth.	Increased antioxidant content and reduced seed water content may contribute to seed survival within the soil seed bank and during germination.	NS	*P* = 0.001, RM = 0.213	NS	*P* = 0.001, RM = 0.280
Biochemistry	Alpha tocopherol (mg/gDW)	α-Toc			NS		NS	
Biochemistry	Total tocopherols (mg/gDW)	Toc			NS		NS	
Biochemistry	Seed water content (%)	WC	Seed water content is related with deterioration and molecular mobility.		*P* = 0.003, RM = 0.145		*P* = 0.003, RM = 0.136	
Biochemistry	Imbibed seed water content (%)	Imb_WC			*P* = 0.001, RM = 0.538		*P* = 0.001, RM = 0.491	

Viability	Percentage of viable embryos (%)	Viability	Account for the percentage of seeds that are viable.	Higher proportion of viable seeds may contribute to increased propagule pressure and increased recruitment, but also contributes to seed survival with time before germination.	P = 0.029, RM = 0.099	P = 0.0.001, RM = 0.239	P = 0.015, RM = 0.093	P = 0.001, RM = 0.170
Viability	Percentage of patchy embryos (%)	Patchy	Account for the percentage seeds with different embryo types with decreased vigor.		P = 0.007, RM = 0.157		P = 0.024, RM = 0.070	
Viability	Percentage of weakly embryos (%)	Weakly			P = 0.007, RM = 0.167		P = 0.008, RM = 0.102	
Viability	Percentage of patchy-weakly embryos (%)	PatchyWeakly			NS		NS	
Viability	Percentage of death embryos (%)	Death			NS		NS	
Viability	Percentage of aborted seeds (%)	Aborted	Account for the percentage of produced seeds that are aborted.		NS		P = 0.009, RM = 0.099	
Viability	Percentage of dying embryos (%)	Dying	Account for the percentage of non-death seeds with different embryo types with decreased vigor.		P = 0.001, RM = 0.232		P = 0.001, RM = 0.167	
Production	Fruit weight (g)	FruitWeight	Define the seed production in terms of weight or number determining propagule pressure.	Higher seed production increases the propagule pressure and potential recruitment.	NS	NS	NS	NS
Production	Total seed weight per fruit (mg)	SeedsWeight			NS		NS	
Production	Total seed number per fruit	SeedNum			NS		NS	
Persistence	Resistance to deterioration (hours)	L_5	Define the seeds potential resistance to ageing.	Long-lived and dormant seeds may allow determine the persistence potential of the soil seed bank.	P = 0.001, RM = 0.247	P = 0.001, RM = 0.245	P = 0.001, RM = 0.182	P = 0.001, RM = 0.192
Persistence	Medium longevity (hours)	L_{50}			P = 0.003, RM = 0.171		P = 0.002, RM = 0.139	
Persistence	Lethal ageing time (hours)	L_{95}			NS		NS	
Persistence	Dormancy percentage (%)	Dormancy	Determine the percentage of the dormant state that may contribute to seed persistence.		NS		NS	
Germination	Total germination percentage (%)	GRP	Determine the percentage of seeds that may germinate when encountering the optimal conditions.	High germination rates and a fast germination increase the	P = 0.042, RM = 0.087	P = 0.008, RM = 0.132	NS	P = 0.020, RM = 0.086
Germination	Mean germination time (days)	MGT	Define the germination timing. Germination timing optimizes the		P = 0.033, RM = 0.094		P = 0.030, RM = 0.073	

Germination	Mean germination rate(seeds/day)	MGR	fitness of seedlings by delivering germinated seeds at the best moment.	potential recruitment.	NS		NS	
Germination	Germination speed percentage (%)	GSP			NS		NS	
Germination	Time required for 50% germination (days)	T_{50}			P = 0.002, RM = 0.162		P = 0.009, RM = 0.125	
Germination	Uncertainty index (bits)	UNC	Define seed germination synchronization and variability. Variability is associated with seedling survival chance in heterogeneous framework.		P = 0.043, RM = 0.094		NS	
Germination	Synchronization index (SYN)	SYN			NS		NS	
Germination	Germination variance	VGT			P = 0.003, RM = 0.188		P = 0.002, RM = 0.124	
Germination	Germination standard deviation	SDG			P = 0.019, RM = 0.112		P = 0.030, RM = 0.080	
Germination	Coefficient of variation (%)	CVG			NS		NS	

Supplementary material

Table S1. Mean values per population and *P*-values of the Zone and Zone (Population) effects in the ANOVA for all measured traits. NS = Not significant. Data is shown as Mean ± SE, N = 5.

		CAP DE CREUS			COSTA BRAVA			MARESME			ANOVA	
Trait Type	**Trait**	**CA1**	**CA2**	**CA3**	**CB1**	**CB2**	**CB3**	**M1**	**M2**	**M3**	**Zone**	**Zone (Population)**
Morphometry	Basal seed area (mm2)	0,89 ± 0,05	0,97 ± 0,05	0,93 ± 0,02	0,89 ± 0,05	1,00 ± 0,02	1,05 ± 0,05	0,81 ± 0,04	0,81 ± 0,03	1,01 ± 0,03	0,021	0,004
Morphometry	Basal seed perimeter (mm)	3,50 ± 0,08	3,64 ± 0,10	3,57 ± 0,05	3,54 ± 0,09	3,71 ± 0,05	3,80 ± 0,07	3,36 ± 0,07	3,32 ± 0,06	3,69 ± 0,06	0,006	0,007
Morphometry	Major basal diagonal (mm)	1,29 ± 0,03	1,33 ± 0,04	1,30 ± 0,03	1,34 ± 0,04	1,38 ± 0,02	1,41 ± 0,02	1,26 ± 0,03	1,21 ± 0,02	1,35 ± 0,02	0,001	0,05
Morphometry	Minor basal diagonal (mm)	0,87 ± 0,03	0,93 ± 0,02	0,91 ± 0,02	0,84 ± 0,02	0,93 ± 0,01	0,95 ± 0,03	0,82 ± 0,02	0,86 ± 0,03	0,94 ± 0,02	**NS**	0,001
Morphometry	Lateral seed area (mm2)	0,55 ± 0,04	0,63 ± 0,03	0,52 ± 0,01	0,56 ± 0,04	0,64 ± 0,00	0,74 ± 0,07	0,47 ± 0,02	0,54 ± 0,02	0,59 ± 0,02	0,003	0,008
Morphometry	Lateral seed perimeter (mm)	3,15 ± 0,11	3,19 ± 0,09	3,06 ± 0,05	3,18 ± 0,11	3,27 ± 0,01	3,46 ± 0,13	2,91 ± 0,04	2,93 ± 0,06	3,25 ± 0,07	0,002	0,03
Morphometry	Major lateral diagonal (mm)	1,35 ± 0,05	1,31 ± 0,05	1,31 ± 0,04	1,39 ± 0,04	1,36 ± 0,01	1,44 ± 0,05	1,26 ± 0,02	1,20 ± 0,03	1,39 ± 0,04	0,007	**NS**
Morphometry	Minor lateral diagonal (mm)	0,52 ± 0,02	0,60 ± 0,02	0,50 ± 0,03	0,51 ± 0,02	0,59 ± 0,00	0,65 ± 0,05	0,48 ± 0,01	0,57 ± 0,01	0,54 ± 0,01	0,036	0,001
Morphometry	Seed mass (mg)	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,03 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,03 ± 0,00	0,02 ± 0,00	0,021	<0.001
Morphometry	Dry seed mass (mg)	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,002	<0.001
Biochemistry	Gamma tocopherol (mg/gDW)	5,80 ± 0,60	5,43 ± 0,44	6,65 ± 0,23	7,55 ± 0,30	5,75 ± 0,47	7,01 ± 0,41	6,27 ± 0,29	6,02 ± 0,28	7,15 ± 0,13	0,037	0,005
Biochemistry	Alpha tocopherol (mg/gDW)	0,23 ± 0,01	0,32 ± 0,02	0,26 ± 0,01	0,28 ± 0,07	0,22 ± 0,01	0,28 ± 0,02	0,28 ± 0,02	0,26 ± 0,01	0,28 ± 0,00	**NS**	**NS**
Biochemistry	Total tocopherols (mg/gDW)	6,03 ± 0,60	5,75 ± 0,45	6,91 ± 0,23	7,83 ± 0,36	5,97 ± 0,46	7,29 ± 0,41	6,55 ± 0,30	6,27 ± 0,28	7,43 ± 0,14	0,045	0,006
Biochemistry	Seed water content (%)	0,13 ± 0,01	0,13 ± 0,00	0,14 ± 0,00	0,13 ± 0,01	0,13 ± 0,01	0,12 ± 0,00	0,14 ± 0,01	0,14 ± 0,01	0,16 ± 0,01	0,002	**NS**
Biochemistry	Imbibed seed water content (%)	0,13 ± 0,01	0,14 ± 0,01	0,15 ± 0,01	0,10 ± 0,01	0,14 ± 0,01	0,16 ± 0,01	0,19 ± 0,01	0,22 ± 0,01	0,23 ± 0,01	<0.001	<0.001
Viability	Percentage of viable embryos (%)	83,57 ± 1,97	77,86 ± 1,42	76,42 ± 2,13	88,72 ± 1,29	81,55 ± 1,99	85,30 ± 1,12	80,15 ± 3,20	71,55 ± 2,41	81,83 ± 1,60	<0.001	<0.001
Viability	Percentage of patchy embryos (%)	6,13 ± 0,85	4,52 ± 0,42	7,33 ± 1,04	4,43 ± 0,96	4,30 ± 1,01	3,23 ± 1,00	12,73 ± 2,38	6,01 ± 1,04	6,38 ± 1,21	<0.001	0,001
Viability	Percentage of weakly embryos (%)	2,99 ± 1,22	5,63 ± 0,93	6,86 ± 2,09	3,13 ± 0,38	4,35 ± 0,34	2,76 ± 1,00	5,13 ± 1,41	11,20 ± 2,25	5,84 ± 1,52	0,005	0,028
Viability	Percentage of patchy-weakly embryos (%)	0,77 ± 0,47	2,26 ± 0,36	0,35 ± 0,35	1,00 ± 1,00	1,11 ± 0,74	0,40 ± 0,40	0,81 ± 0,50	0,39 ± 0,39	0,75 ± 0,75	**NS**	**NS**
Viability	Percentage of death embryos (%)	4,70 ± 1,13	4,85 ± 1,48	4,82 ± 1,98	2,37 ± 0,66	4,70 ± 0,85	4,70 ± 1,25	0,79 ± 0,49	10,47 ± 1,96	3,96 ± 1,12	**NS**	0,001
Viability	Percentage of aborted seeds (%)	1,84 ± 0,83	4,88 ± 0,96	4,21 ± 1,73	0,36 ± 0,36	4,00 ± 1,05	3,62 ± 1,17	0,38 ± 0,38	0,38 ± 0,38	1,23 ± 0,50	0,001	0,032
Viability	Percentage of dying embryos (%)	9,88 ± 2,32	12,41 ± 0,88	14,55 ± 1,90	8,55 ± 0,93	9,76 ± 1,05	6,38 ± 1,19	18,68 ± 3,37	17,60 ± 1,95	12,98 ± 2,34	<0.001	**NS**
Production	Fruit weight (g)	2,07 ± 0,29	0,95 ± 0,16	1,94 ± 0,31	2,74 ± 0,33	1,71 ± 0,19	0,61 ± 0,08	1,97 ± 0,35	1,30 ± 0,24	1,48 ± 0,25	**NS**	<0.001
Production	Total seed weight per fruit (mg)	0,62 ± 0,06	0,10 ± 0,04	0,57 ± 0,10	1,07 ± 0,14	0,42 ± 0,06	0,03 ± 0,02	0,71 ± 0,16	0,36 ± 0,07	0,46 ± 0,13	**NS**	<0.001
Production	Total seed number per fruit	1371,19 ± 132,81	208,33 ± 76,44	1224,46 ± 199,84	2478,45 ± 345,32	746,73 ± 113,60	57,76 ± 42,80	1851,21 ± 445,77	718,62 ± 133,55	945,61 ± 274,28	**NS**	<0.001
Persistence	Resistance to deterioration (hours)	31,07 ± 0,99	43,92 ± 0,73	46,15 ± 3,65	45,67 ± 1,24	41,96 ± 2,38	39,27 ± 3,43	45,90 ± 0,96	8,08 ± 1,37	31,68 ± 3,62	<0.001	<0.001
Persistence	Medium longevity (hours)	52,39 ± 0,37	48,80 ± 0,76	47,97 ± 7,26	57,29 ± 1,26	58,32 ± 1,36	52,28 ± 1,18	51,95 ± 0,81	29,74 ± 1,82	48,65 ± 0,94	<0.001	<0.001
Persistence	Lethal ageing time (hours)	70,42 ± 2,78	63,10 ± 5,92	77,22 ± 31,63	92,88 ± 1,99	91,28 ± 7,85	66,03 ± 4,30	73,45 ± 2,19	75,11 ± 5,81	76,35 ± 7,11	**NS**	**NS**
Persistence	Dormancy percentage (%)	56,08 ± 1,66	35,06 ± 2,11	28,45 ± 2,94	33,64 ± 4,35	55,15 ± 3,29	14,90 ± 4,61	4,44 ± 2,31	49,65 ± 2,37	46,22 ± 2,87	0,035	<0.001
Germination	Total germination percentage (%)	27,50 ± 2,87	42,80 ± 1,62	47,98 ± 2,20	55,08 ± 4,53	26,40 ± 2,32	70,80 ± 4,32	79,90 ± 2,28	21,90 ± 4,09	35,61 ± 1,90	<0.001	<0.001
Germination	Mean germination time (days)	35,75 ± 2,92	34,14 ± 2,86	31,14 ± 1,86	39,08 ± 0,88	32,32 ± 2,25	14,15 ± 0,65	42,70 ± 1,47	42,22 ± 4,12	37,62 ± 2,55	<0.001	<0.001
Germination	Mean germination rate(seeds/day)	0,03 ± 0,00	0,03 ± 0,00	0,03 ± 0,00	0,03 ± 0,00	0,03 ± 0,00	0,07 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,03 ± 0,00	<0.001	<0.001
Germination	Germination speed percentage (%)	2,87 ± 0,23	3,02 ± 0,27	3,26 ± 0,19	2,56 ± 0,06	3,16 ± 0,24	7,13 ± 0,36	2,35 ± 0,08	2,47 ± 0,26	2,71 ± 0,19	<0.001	<0.001
Germination	Uncertainty index (bits)	2,70 ± 0,19	3,24 ± 0,16	3,09 ± 0,08	2,77 ± 0,06	2,82 ± 0,12	2,65 ± 0,11	3,27 ± 0,12	2,22 ± 0,23	2,87 ± 0,11	**NS**	<0.001
Germination	Synchronization index (SYN)	0,11 ± 0,02	0,08 ± 0,02	0,10 ± 0,01	0,15 ± 0,01	0,08 ± 0,01	0,17 ± 0,02	0,10 ± 0,01	0,15 ± 0,02	0,11 ± 0,01	0,007	0,002
Germination	Germination variance	735,17 ± 128,94	642,01 ± 109,62	519,47 ± 61,83	795,04 ± 37,07	596,94 ± 85,37	99,27 ± 9,48	977,77 ± 75,60	1137,97 ± 226,20	785,74 ± 108,07	<0.001	<0.001
Germination	Germination standard deviation	26,69 ± 2,38	24,94 ± 2,24	22,63 ± 1,35	28,16 ± 0,67	24,15 ± 1,87	9,91 ± 0,50	31,18 ± 1,20	32,95 ± 3,62	27,74 ± 2,00	<0.001	<0.001
Germination	Coefficient of variation (%)	74,49 ± 0,61	72,91 ± 0,66	72,67 ± 0,21	72,06 ± 0,23	74,52 ± 0,96	70,02 ± 0,51	72,98 ± 0,42	77,60 ± 1,62	73,66 ± 0,35	0,001	<0.001
Germination	Time required for 50% germination (days)	31,99 ± 2,77	28,28 ± 2,87	23,60 ± 2,75	38,18 ± 1,49	27,58 ± 3,20	10,69 ± 0,37	40,49 ± 1,43	37,88 ± 6,77	36,66 ± 3,37	<0.001	<0.001

Capítol 4. Plasticitat en la resposta hormonal a l'estrès per baixes temperatures de la planta invasora *Carpobrotus edulis*

Chapter 4. Plasticity in the hormonal response to cold stress in the invasive plant *Carpobrotus edulis*

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Plasticity in the hormonal response to cold stress in the invasive plant *Carpobrotus edulis*

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ABSTRACT

Cold stress response is mediated by multiple signaling pathways with complex interactions, among which phytohormones may play a role. We explored changes in the contents of phytohormones, including abscisic acid, jasmonic acid, salicylic acid, auxin, cytokinins, gibberellins and melatonin, along with stress tolerance markers in an invasive halophyte, *Carpobrotus edulis* in response to chilling. In a first experiment, plants were exposed to mean daily temperatures from 10 °C to 5 °C during a cold wave in an experimental garden. In a second experiment, plants were subject to slowly decreasing temperatures, from 20 to 5 °C, in a climatic chamber. Although the cold response in both experiments was associated with a similar extent of leaf desiccation, hormonal variations differed. Cold stress reduced melatonin contents, while it increased salicylic acid contents in the experimental garden. Rather, transient increases in the contents of melatonin occurred in parallel with sustained increases in the contents of abscisic acid and cytokinins in the climatic chamber. In both experiments, plants were able to prevent cold-induced increases in lipid peroxidation and any eventual damage to the photosynthetic apparatus. We conclude that (i) the hormonal response to chilling in *C. edulis* is strongly dependent on time exposure to low temperatures, severity of stress, as well as other environmental conditions, (ii) the hormonal response of this plant species to low temperatures is very plastic, thus underlining its great capacity for cold acclimation.

1. Introduction

Among multiple factors that threat ecosystems productivity, low temperatures constitute one of the main constrains to plant growth, development and distribution, causing plant stress, damage and even productivity loss (Sanghera et al., 2011). Plants have developed several mechanisms to face stressful factors such as salinity, drought, or extreme temperature, among others; and some cold stress response mechanisms have been shown to be almost ubiquitous in the plant kingdom, while others are strongly species specific (Zhang and Sonnewald, 2017). Plant responses to low temperatures are complex as they induce changes at multiple organization levels that include morphological, physiological and biochemical adjustments, as well as alterations in gene expression (John et al., 2016). Almost half of the modern angiosperms inhabit temperate climates, suggesting that cold tolerance may be a difficult trait to evolve (Preston and Sandve, 2013).

Temperature determines biological thermodynamic processes, including biomolecule stability and function, and therefore it is crucial for optimal cellular processes and homeostasis. However, cold injury strongly differs whether temperatures range between 0–15 °C (chilling) or below the water melting point involving ice formation (freezing) (Levitt, 1980). Under natural conditions, plant species in both the cold temperate zones and the tropical highlands experience chilling as part of seasonality events. One of the first cold adaptation responses is to escape low temperatures through endodormancy or other changes in phenology allowing a displacement of sensitive development stages such as reproduction (Preston and Sandve, 2013). When exposure to low temperatures cannot be avoided, however, chilling induces reduced leaf expansion, wilting, chlorosis, long-distance water transport arrest and eventually cell, organ and plant death, depending on the duration and severity of the stress and the occurrence of other prevailing stress factors (John et al., 2016).

Cold acclimation is the phenomenon through which plants show increased cold tolerance during exposure to low temperatures, in a process that involves extensive reprogramming of gene expression and metabolism (Megha et al., 2018). Generally, cold stress leads to tissue

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Abbreviations: ABA, abscicic acid; Chl, chlorophyll; *Fv/Fm* ratio, maximum efficiency of photosystem II; JA, jasmonic acid; LWC, leaf water content; PSII, photosystem II; SA, salicylic acid

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desiccation and increases in proteins and compounds with various protective functions (antioxidants, biomolecule stabilizers and osmoregulators); and major metabolic pathways (*e.g.* photosynthesis) are suppressed leading to growth arrest (John et al., 2016). To withstand chilling, cold-tolerant plants modify membrane lipid content and increase the proportion of unsaturated fatty acids to maintain lipid membrane fluidity (Khodakovskaya et al., 2006). An imbalance between energy absorption and its use for photochemical pathways leads to the formation of reactive oxygen species (ROS), which gives rise to an increase in antioxidants in tolerant species (John et al., 2016). Furthermore, ROS signaling may interact with abscisic acid (ABA) to provide tolerance to cold stress in several species (Megha et al., 2018). In spite of the fact that several studies have tried to understand hormonal responses to cold, to our knowledge no studies have considered thus far a complete hormonal profiling approach, particularly in non-model species with a particular interest in invasion biology. In the present study, we aimed to get new insights into our understanding of (i) the hormonal and physiological response to chilling in the invasive plant, *C. edulis*, (ii) the plasticity in the response of this plant species to low temperatures under different climatic conditions.

2. Material and methods

2.1. Study species and experimental design

Carpobrotus edulis (L.) N.E.Br. is a mat-forming succulent clonal plant belonging to the Aizoaceae family, commonly used as a dune-fixer and also highly valorized as an exotic ornamental plant. Those may be the reasons why it was introduced from South Africa and became invasive in several Mediterranean regions, such as some parts of California and Australia, and the Mediterranean basin (D'Antonio and Mahall, 1991). *C. edulis* grows vigorously in its invaded range expanding its distribution into a wide range of temperatures. Seeds of *C. edulis* were collected in a protected area in Natural Park of Cap de Creus in northeast Spain (42°21′N, 3°11′E) where this species is showing an important expansion as an invasive species (Fenollosa et al., 2016). Dehydrated fruits from different *C. edulis* clumps (at least 10 m apart) were collected and stored at 4 °C until germination. On May 2016, after seed disinfection with commercial bleach, seeds germinated under a long light cycle (photoperiod of 16 h/8 h light:darkness), 21 °C and 65% humidity. During September 2016, *C. edulis* individuals were transferred into 1 L pots in the greenhouse to ensure enough plant material for the experiments.

For experiment 1, cold acclimation was evaluated in plants growing under natural climatic conditions in an experimental garden. Six similar potted-grown individuals were put outside from the greenhouse into the experimental garden of the Faculty of Biology (Barcelona, NE Spain) during December 2016. The experiment was conducted from the 29th of December 2016 to the 27th of February 2017 under Mediterranean winter conditions. Plants were sampled at five different time points, from December 2016 to March 2017. During January, a cold wave (cold shock) was alerted and plants were sampled just before the cold wave (2nd sampling point – 13 January), during it (3rd sampling point – 17 January), just after it (4th sampling point – 24 January) and one month later (5th sampling point – 27 February) (Fig. 1). All selected days were clear days and sampling was always performed at noon (12:00 solar time) with a photosynthetically-active photon flux density ranging between 1000 and 1400 μmol s^{-1} m^{-2}.

For experiment 2, plants were kept in the greenhouse from September 2016 to January 2017, when chambers were ready for the experiment under controlled climatic conditions. This experiment started during February 2017 using two identical controlled chambers (Ibercex model E-1350-DV, Madrid, Spain) running in parallel under the following climatic conditions (temperature = 21 °C, relative humidity = 65%, photosynthetically-active photon flux density = 380 μmol s^{-1} m^{-2}, photoperiod of 12 h/12 h light/darkness). After three months of light acclimation, one chamber temperature was kept around 21 °C whereas the other was set to progressive decreases of approximately 5 °C every 7 days. Samplings were performed in the middle of the photoperiod at 21 °C, 15 °C, 8.6 °C and 5.3 °C just after acclimation for 7 days for each temperature (Fig. 1).

For both experiments, one fully-developed young leaf per plant was used for the measurements of cold stress tolerance markers, while two additional leaves (at similar developmental stage) per plant were pooled, immediately frozen in liquid nitrogen and stored at −80 °C for later biochemical analyses, including measurements of chlorophylls and lipid peroxidation, melatonin and hormone profiling.

2.2. Cold stress tolerance markers

For both experiments, a leaf per individual was taken to immediately measure its area (through image analysis performed with ImageJ Software, NIH, Bethesda, USA), fresh weight (FW), and dry weight (DW) (over-drying the tissue for 48 h at 60 °C until constant weight). Leaf water content was determined as LWC = (FW-DW)/DW. After 30 min of dark acclimation, the maximum efficiency of the photosystem II (PSII, F_v/F_m ratio) was measured with the MINI-PAM photosynthesis yield analyzer (Walz, Effeltrich, Germany) as described previously (Bilger et al., 1995).

2.3. Chlorophylls and lipid peroxidation

Leaf samples were ground in liquid nitrogen using a mix ball and extracted with cold methanol containing 0.01% butylated hydroxytoluene using ultrasonication. After centrifuging at 12,000 rpm for 10 min at 4 °C, the supernatant was collected and the pellet re-extracted with the same solvent until it was colorless. Supernatants were pooled and chlorophylls measured spectrophotometrically following Lichtenthaler and Wellburn, 1983. The extent of lipid peroxidation, which was used as an oxidative stress marker, was estimated using the same methanolic extracts by measuring the accumulation of lipid hydroperoxides following the modified ferrous oxidation-xylenol orange (FOX) assay as described in DeLong et al. (2002).

2.4. Melatonin and hormone profiling

Leaf samples were ground in liquid nitrogen using a mix ball and extracted with methanol:isopropanol:acetonitrile (50:49:1) using ultrasonication. After centrifuging at 12,000 rpm for 10 min at 4 °C, the supernatant was collected and the pellet re-extracted with the same solvent until it was colorless. Supernatants were pooled and filtered with 0.22 μm PTFE filters (Phenomenex, Torrance, USA), transferred to HPLC vials and injected into the UHPLC-MS/MS. Melatonin and phytohormones, including ABA, salicylic acid (SA), jasmonic acid (JA), the auxin, indole-3-acetic acid, the cytokinins *trans*-zeatin and *trans*-zeatin riboside, and gibberellin 4 (GA_4), were separated using an elution gradient on a reverse-phase UHPLC system and quantified using tandem mass spectrometry in multiple reaction monitoring mode. Recovery rates were calculated for each hormone on every sample by using deuterated compounds as described by Müller and Munné-Bosch (2011).

2.5. Statistical analyses

For experiment 1, a one-way mixed ANOVA of repeated measures with 'time' as within-samples factor was performed. For experiment 2, a two-way mixed ANOVA of repeated measures with 'treatment' (cold acclimated *vs.* control) as between-samples factor and 'temperature' (different time points) as a within-samples factor was performed. Tuckey test was used as a post-hoc method. All analyses were performed through the *nlme* and *multcomp* packages in R 3.3.3.

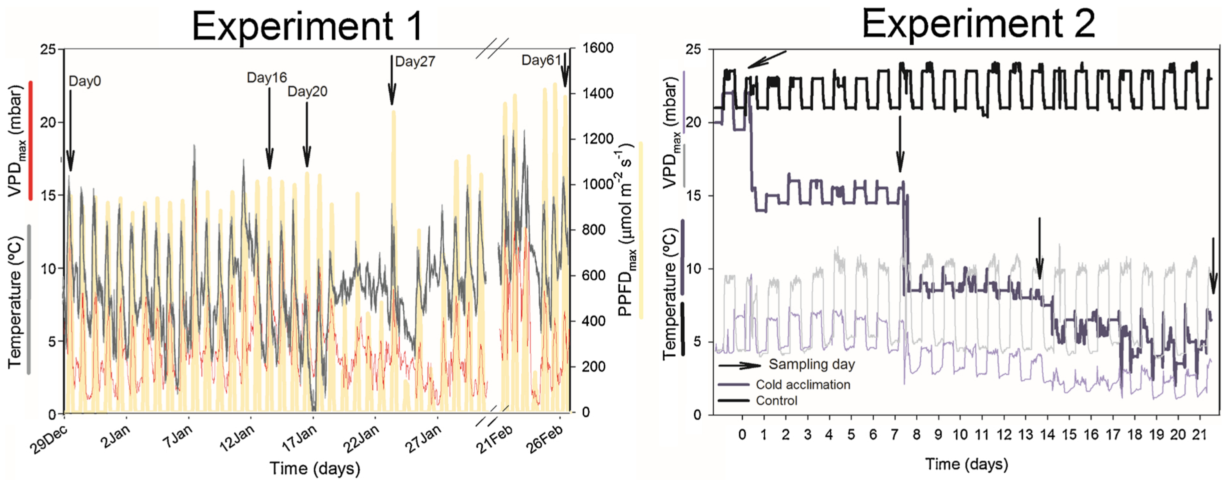

Fig. 1. Meteorological conditions for both experiments. Arrows represent sampling days, whose meteorological conditions are described in Table 1. VPD$_{max}$ = maximum vapor pressure deficit, PPFD$_{max}$ = photosynthetically-photon flux density. As the cold acclimation experiment was performed in controlled chambers, radiation was constant around 380 µmol s^{-1} m^{-2}. Data for experiment 1 was provided by *Servei meteorologic de Catalunya* from the nearest automatic station situated at 200 m from the experimental plot, whereas data for experiment 2 was registered with a data logger (EL-USB-2-LCD, Lascar Electronics, Whiteparish, UK) inside each chamber taking measures every 30 min.

3. Results

3.1. Cold stress tolerance in *C. edulis*

In the first experiment (experimental garden), plants were exposed to diurnal cycles of light/darkness and therefore to realistic conditions of cold stress in a maritime Mediterranean winter, in this particular case with minimum temperatures very close to freezing (1 °C) during 17 January, that is 20 days from the start of the experiment (Table 1). During experiment 2 (controlled, climatic chambers), plants were gradually left to acclimate to stepwise decreases in temperature (5 °C every week) for a period of 3 weeks, reaching also minimum temperatures very close to freezing (2 °C) at 21 days from the start of the experiment (Table 1). Photosynthetically-active photon flux density to which plants were exposed was lower and relative humidity higher in the second experiment compared to the first one (Table 1).

Leaf hydration, as estimated by the leaf water content (LWC), decreased in both experiments by *ca.* 30% (Fig. 2). Cold-induced dehydration was similar in both experiments, with LWCs at maximum cold stress of 6.3 gH_2O/g DW and 5.9 gH2O/g DW in the experimental garden and climatic chamber, respectively, showing no significant differences between them (Student's t-test, $P > 0.05$). Chl $a + b$ contents also decreased during experiment 1 by *ca.* 30%, but they did not recover to initial pre-cold stress values after stress release (Fig. 2). Indeed, the Chl a/b ratio increased 30% during the period of maximum stress and it did not recover either (Fig. 2). By contrast, Chl $a + b$ contents and the Chl a/b ratio kept unaltered during cold stress exposure in the climatic chamber (experiment 2). While the extent of lipid peroxidation, as indicated by lipid hydroperoxide contents, was unaltered in the experimental garden, it decreased by 35% at maximum stress in the climatic chamber (Fig. 2). The F_v/F_m ratio remained constant and always above 0.75 in both experiments (data not shown).

3.2. Melatonin and hormone profiling

Melatonin contents ranged between 2 and 1100 ng/g DW in leaves of *C. edulis* plants during both experiments, which corresponds to maximum values of 1.1 µg/g DW (Fig. 3). Although cold stress led to significant increases in endogenous melatonin contents at 14 days of cold stress exposure in the cold chamber, further decreases in

Table 1

Meteorological conditions for the sampling days during the two experiments. For the first experiment, data was provided by *Servei meteoròlogic de Catalunya* from the nearest weather station situated at 200 m from the experimental garden (Barcelona, NE Spain), whereas data for the second experiment was registered with a data logger inside each chamber taking measures every 30 min. VPD$_{max}$ = maximum vapor pressure deficit, GSR$_{max}$ = maximum global solar radiation. Light and dark blue colors indicate temperatures between 5–10 °C and 3–5 °C, respectively, while purple colors indicate temperatures between 0–2 °C. (For interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

	Experiment 1					Experiment 2				
Sampling day	29 Dec	13 Jan	17 Jan	24 Jan	27 Feb	Control	Cold chamber			
Experimental days	0	16	20	27	61	0,7,14,21	0	7	14	21
Sampling temp (°C)	14.6	12.4	7.8	10.1	15.6	21.0	21.0	15.0	8.6	5.3
Min Temp. (°C)	4.6	4.7	1.0	5.8	9.6	20.5	19.5	14.0	7.5	2.0
Mean Temp. (°C)	10.3	10.6	4.7	8.6	12.6	21.0	21.0	15.0	8.6	5.3
Max Temp. (°C)	16.4	13.7	7.8	14.5	16.2	23.5	22.0	16.5	10	8.5
Relative hum (%)	54	45	32	56	71	72	64	68	70	76
VPD$_{max}$ (KPa)	1.3	1.0	0.8	0.8	0.7	1.1	1.2	0.7	0.4	0.3
PPFD$_{max}$ (µmol m^{-2} s^{-1})	950	1010	1044	1319	1373	380	380	380	380	380

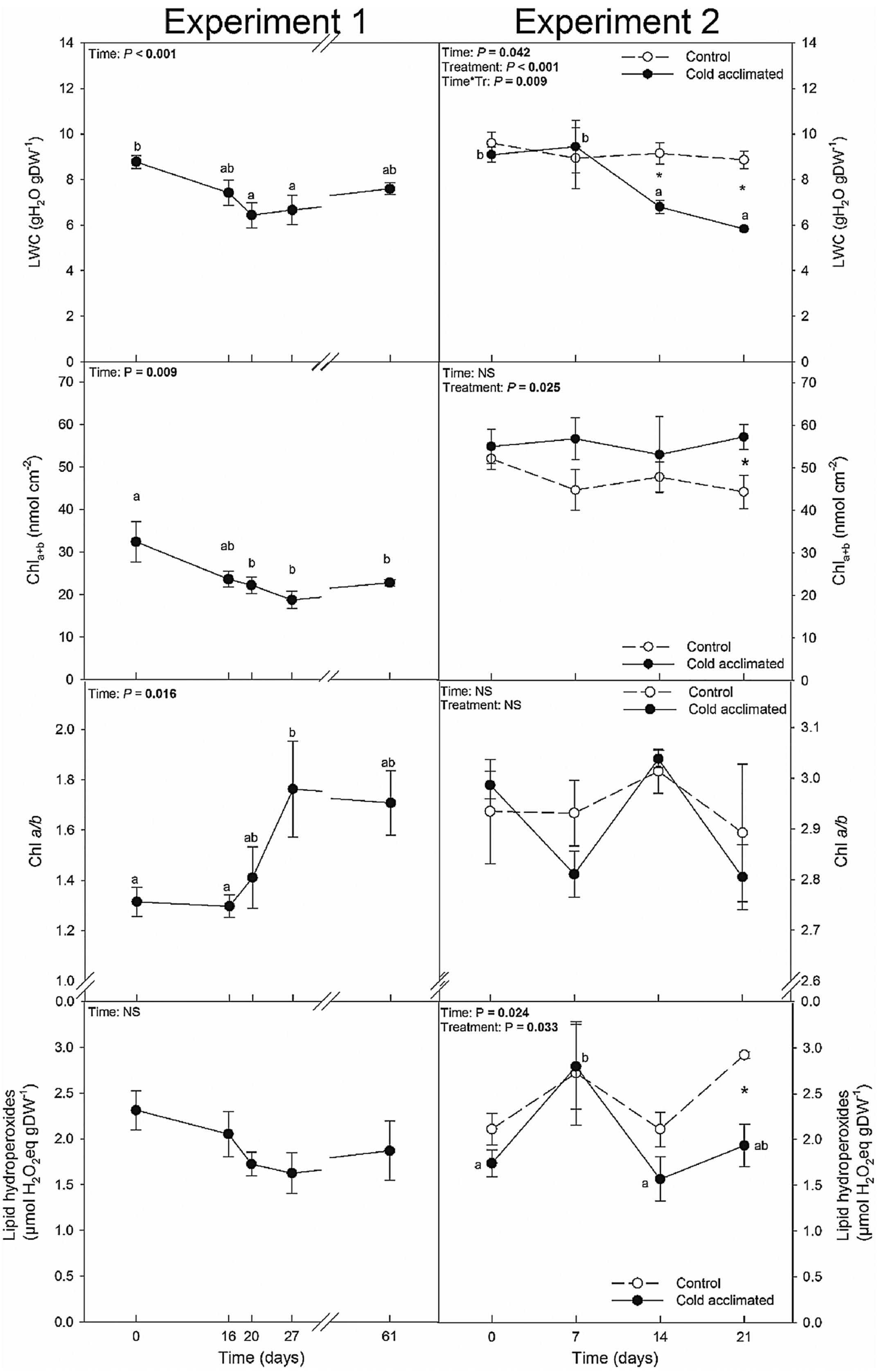

Fig. 2. Leaf water content (LWC), total chlorophyll (Chl) contents, Chl *a/b* ratio and lipid hydroperoxide contents in *Carpobrotus edulis* exposed to cold stress during both experiments. The temperatures registered during the experiment and for each sampling point are shown in Fig. 1 and Table 1, respectively. Data are the mean ± SE from 5 to 6 individuals. Different letters represent differences among sampling times and asterisks differences between treatments in a given time.

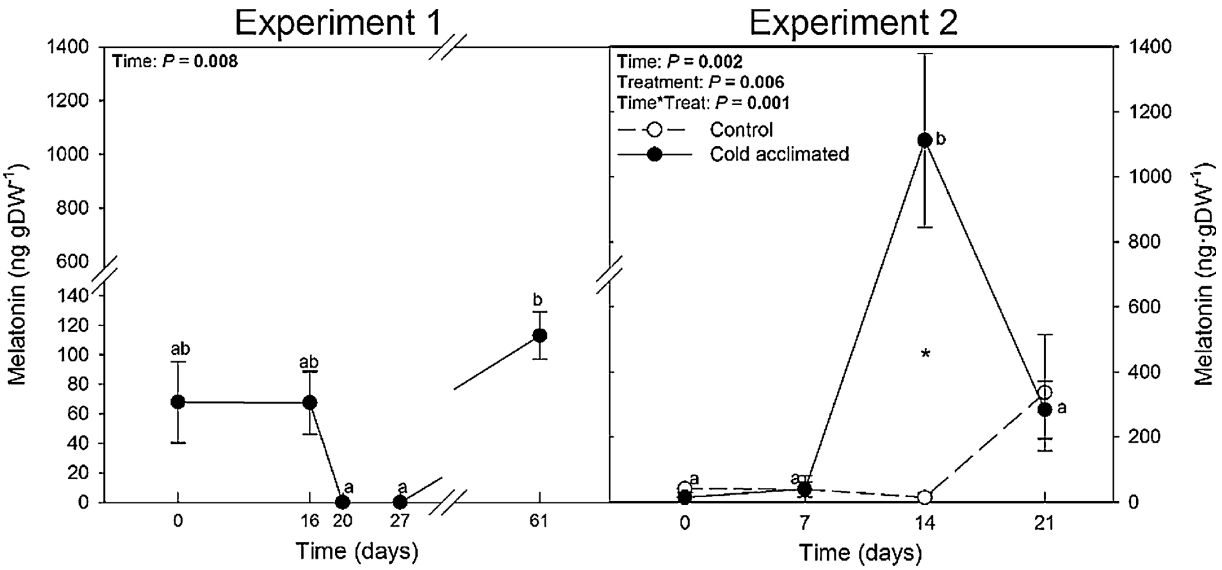

Fig. 3. Endogenous melatonin contents in *Carpobrotus edulis* exposed to cold stress during both experiments. The temperatures registered during the experiment and for each sampling point are shown in Fig. 1 and Table 1, respectively. Data are the mean ± SE from 5 to 6 individuals. Different letters represent differences among sampling times and asterisks differences between treatments in a given time.

temperature led to sharp decreases in the contents of this compound to values similar to controls after 21 days of cold treatment (Fig. 3). Interestingly, the minimum melatonin contents were also attained at maximum stress (days 20 and 27) during experiment 1 (Fig. 3).

Melatonin contents (Fig. 3) were in the same order of magnitude than those of stress-related phytohormones, such as ABA, SA and JA (Fig. 4). Cold-induced variations in melatonin (Fig. 3) were however completely different to those shown by ABA, SA and JA (Fig. 3). ABA contents kept unaltered during experiment 1, and increased significantly upon cold stress exposure during experiment 2, doubling values at days 14 and 21 in the cold treatment compared to controls in the climatic chamber (Fig. 4). ABA contents were 5-fold higher at the start of the experiment in the experimental garden compared to the climatic chamber, but they did not increase upon cold stress exposure during experiment 1 (Fig. 4). SA contents increased at day 27 to not recover later in the experimental garden and JA contents were lower at day 21 in cold-exposed plants compared to controls during experiment 2 (Fig. 4). The endogenous contents of *t*Z and its precursor *t*ZR increased in response to cold stress in the climatic chamber at day 21 to keep high levels upon severe stress (Fig. 5), while *t*Z and *t*ZR contents were not altered in the experimental garden (Fig. 5). Endogenous contents of indole-3-acetic acid, and GA_4 kept unaltered throughout both experiments in *C. edulis* leaves (data not shown).

4. Discussion

4.1. Common and differential hormonal responses to cold stress in C. edulis

Although similar in the magnitude of cold to which plants were exposed, the two experiments illustrate some common and differential features in the hormonal response to low temperature stress in *C. edulis*. This plant species is an invasive plant that grows particularly well in maritime Mediterranean areas with temperature reaching close or occasionally a few degrees below 0 °C under natural field conditions (Fenollosa et al., 2017). Plants showed a similar degree of leaf dehydration and were equally effective at preventing damage to PSII upon cold exposure during both experiments. However, the mechanisms used to achieve this similar degree of resistance differed in the two experiments. In the first experiment, the cold stress response was characterized by increases in both the Chl *a/b* ratio and endogenous SA contents, which did not recover to initial values after cold stress exposure. In the second experiment, melatonin contents increased sharply at 21 days of cold stress, to decrease later upon severe stress; and this response was accompanied by sustained increases of ABA and cytokinins. These differences may be attributed to a number of factors. Moderately high solar radiation in the experimental garden together with sudden exposure to low temperatures during the cold wave (experiment 1) might have caused plastic changes in photosynthesis-related processes, such as reductions in the pigment antennae per reaction center, as indicated by higher Chl *a/b* ratios (Kirst et al., 2012). Furthermore, increases in the endogenous salicylic acid contents may help keep photosynthetic activity under these environmental conditions (Scott et al., 2004; Soliman et al., 2018). In contrast, a more gradual exposure to cold stress may have resulted in a differential hormonal response during experiment 2. In this experiment, plants showed enhanced melatonin, ABA and cytokinins contents. Cold-induced increases in ABA have already been reported in other species as a mechanism to prevent leaf dehydration and induce the accumulation of osmoprotectants (Huang et al., 2017); and enhanced cytokinin contents may help maintain chloroplast integrity under stress in the growth chamber (Cela et al., 2018). Furthermore, lower light intensities during experiment 2 compared to experiment 1 may also help explain the lack of response in the Chl *a/b* ratio (no need to reduce antennae, see Kirst et al., 2012).

Interestingly, these two completely differential physiological and hormonal responses led to the same result in terms of cold stress acclimation in *C. edulis*, as indicated by the maintenance of F_v/F_m ratios above 0.75 and lack of cold-induced lipid peroxidation. These results illustrate the resistance of this plant species to cold stress and more particularly a significant plasticity in the plant hormonal response to cold stress depending on the other prevailing environmental conditions. It is also interesting to note that in both experiments, ABA contents either increased in response to cold stress (experiment 2) or were already constitutively very high (experiment 1), thus suggesting high ABA contents is a common hallmark of the cold stress response in *C. edulis*.

4.2. Melatonin cross-talk with other phytohormones

Melatonin (N-acetyl-5methoxytriptamine) is generally considered to be a low-molecular-weight antioxidant in plants due to its activity against ROS *in vitro* (Reiter et al., 2001). However, it has also been proposed to act as a key plant growth regulator, in analogy to its role in

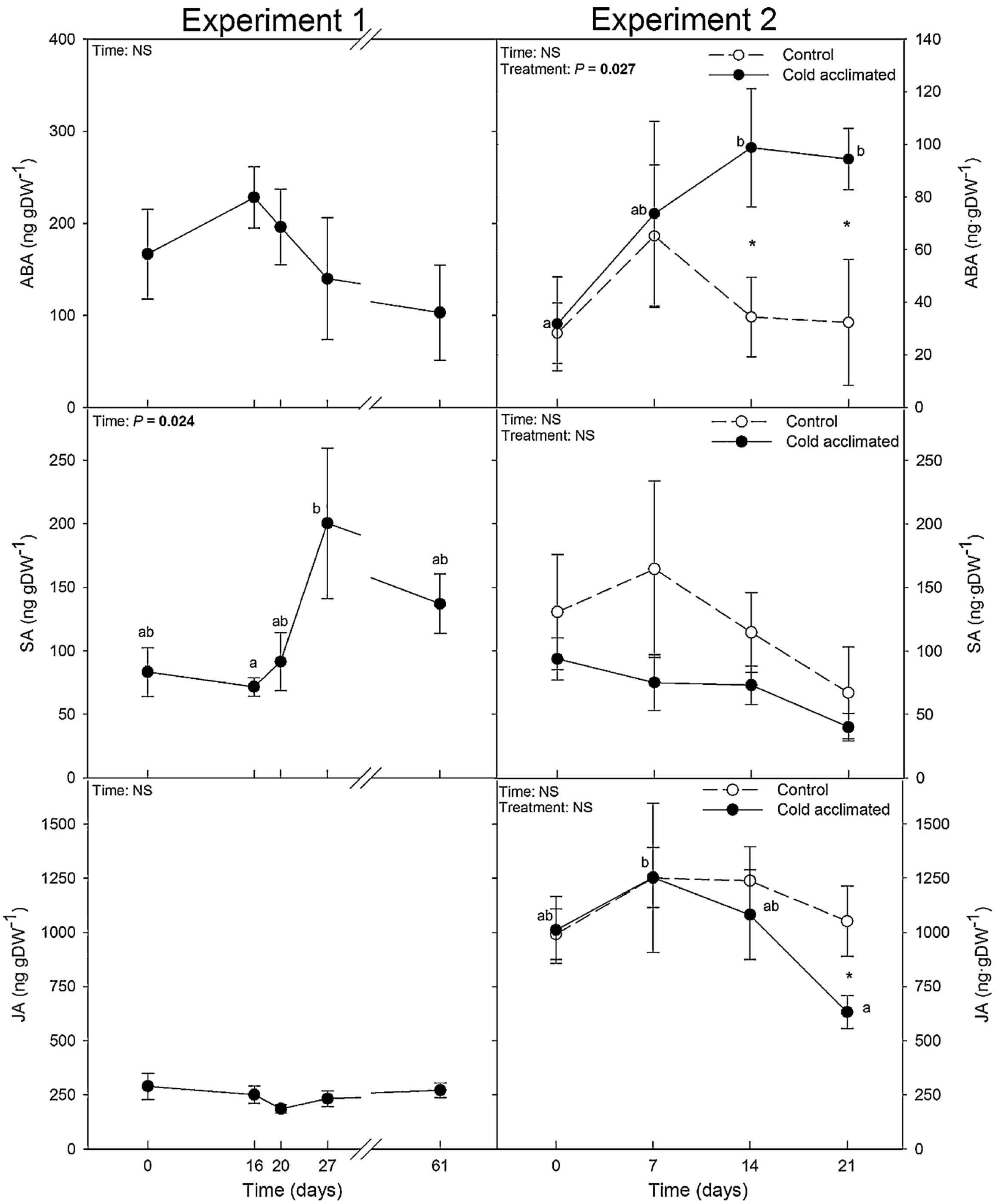

Fig. 4. Endogenous contents of abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) in *Carpobrotus edulis* exposed to cold stress during both experiments. The temperatures registered during the experiment and for each sampling point are shown in Fig. 1 and Table 1, respectively. Data are the mean ± SE from 5 to 6 individuals. Different letters represent differences among sampling times and asterisks differences between treatments in a given time.

circadian control in humans (Tan et al., 2012; Zhang et al., 2014). However, evidence for these functions has been obtained in a very limited number of species only. The endogenous amounts of a compound can determine whether or not this can act as antioxidant or as a phytohormone, and endogenous changes may well inform about the putative role of a compound as an antioxidant or a hormone (Arnao and Hernández-Ruiz, 2014; Fleta-Soriano et al., 2017). Melatonin was found at low concentrations in leaves of *C. edulis* (ranging between 2 and 1100 ng/g DW), which are within the order of magnitude of a phytohormone or regulator, rather than an antioxidant (Arnao and Hernández-Ruiz, 2014, Fleta-Soriano et al., 2017). Melatonin contents were very low and *C. edulis* already contains other antioxidants found at much higher concentrations in chloroplasts (Fenollosa et al., 2017), so a direct antioxidant role for melatonin, presumably thought to be located in chloroplasts and mitochondria (Tan et al., 2013), is at best marginal, as it has been suggested for other plant species (Fleta-Soriano et al., 2017). Recent studies have shown that melatonin may upregulate the expression of the gene encoding isopentenyl transferase (IPT, Ma et al., 2018), a key regulatory enzyme that is essential for cytokinin biosynthesis (Kant et al., 2015). Therefore, melatonin increases at 14 days of cold acclimation in the climatic chamber might result in increases in cytokinin contents in *C. edulis*. However, cytokinin contents kept high

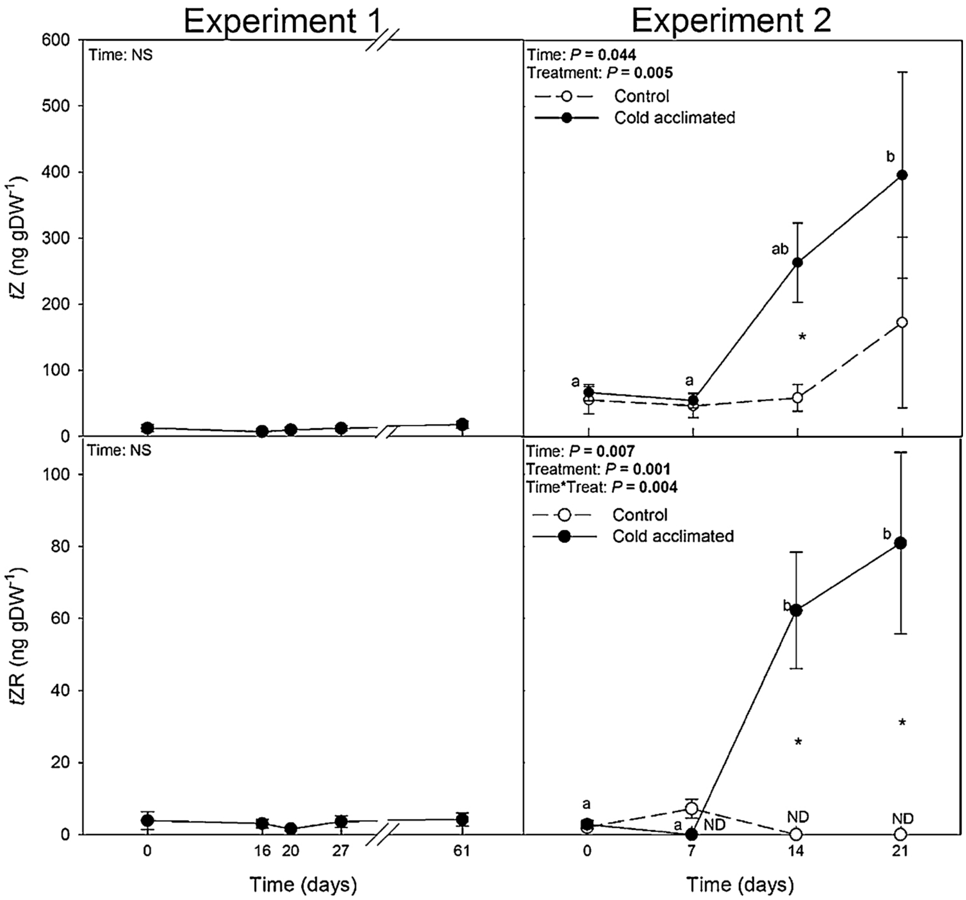

Fig. 5. Endogenous contents of *trans*-zeatin (*t*Z) and its precursor, *trans*-zeatin riboside (*t*ZR) in *Carpobrotus edulis* exposed to cold stress during both experiments. The temperatures registered during the experiment and for each sampling point are shown in Fig. 1 and Table 1, respectively. Data are the mean ± SE from 5 to 6 individuals. Different letters represent differences among sampling times and asterisks differences between treatments in a given time.

at 21 days of cold stress, while melatonin decreased, thus indicating that the melatonin-cytokinin interaction, if any, might have occurred transiently, and not sustained on time during acclimation to low temperatures in the growth chamber. It has also been shown that exogenous melatonin may upregulate ABA-related biosynthesis genes and down-regulate the catabolism of this hormone in cucumber seedlings (Zhao et al., 2017). Therefore, a possible cross-talk of melatonin with ABA and cytokinins in cold stress acclimation in *C. edulis* is possible, although additional investigation is required to better understand the role of melatonin and its interaction with other hormones in cold acclimation in this species. Interestingly, high constitutive ABA contents did not occur concomitantly with high melatonin and cytokinin contents during the plant response to sudden exposure to cold stress in the experimental garden.

It is concluded that the hormonal and physiological response to chilling in *C. edulis* is not only dependent on the time of exposure to low temperatures and severity of stress, but also on the other prevailing environmental conditions to which plants are exposed to. However, the response of *C. edulis* to low temperatures was very plastic, thus underlining the great capacity of this species to acclimate to chilling. While the cold-induced hormonal response in the experimental garden was characterized by enhanced SA contents; melatonin and cytokinin contents increased in response to low temperature stress in the climatic chamber. In both experiments, ABA contents increased (growth chamber) or they were already very high constitutively (experimental garden), thus suggesting ABA has a central role in the response of this plant species to cold stress. Overall, the results are indicative of the extraordinary plasticity of the hormonal response of *C. edulis* to cold stress.

Author contributions

E.F. and S.M.B. designed the study; E.F. and A.G. performed the physiological measurements; E.F. performed the statistical analysis; E.F. and S.M.B. contributed to the interpretation of results; E.F. and S.M.B. wrote the manuscript.

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References

Arnao, M.B., Hernández-Ruiz, J., 2014. Melatonin: Plant growth regulator and/or biostimulator during stress? Trends Plant Sci. 19, 789–797.

Bilger, W., Schreiber, U., Bock, M., 1995. Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. Oecologia 102, 425–432.

Cela, J., Tweed, J.K.S., Sivakumaran, A., Lee, M.R.F., Mur, L.A.J., Munné-Bosch, S., 2018. An altered tocopherol composition in chloroplasts reduces plant resistance to *Botrytis cinerea*. Plant Physiol. Biochem. 127, 200–210.

D'Antonio, C.M., Mahall, B.E., 1991. Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. Am. J. Bot. 78, 885–894.

DeLong, J.M., Prange, R.K., Hodges, D.M., Forney, C.F., Bishop, M.C., Quilliam, M., 2002. Using a modified ferrous oxidation-xylenol orange (FOX) assay for detection of lipid hydroperoxides in plant tissue. J. Agric. Food Chem. 16, 248–254.

Fenollosa, E., Roach, D.A., Munné-Bosch, S., 2016. Death and plasticity in clones influence invasion success. Trends Plant Sci. 21, 551–553.

Fenollosa, E., Munné-Bosch, S., Pintó-Marijuan, M., 2017. Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*. Physiol. Plant. 2, 185–200.

Fleta-Soriano, E., Díaz, L., Bonet, E., Munné-Bosch, S., 2017. Melatonin may exert a protective role against drought stress in maize. J. Agron. Crop Sci. 203, 286–294.

Huang, X., Shi, H., Hu, Z., Liu, A., Amombo, E., Chen, L., Fu, J., 2017. ABA is involved in regulation of cold stress response in Bermudagrass. Front. Plant Sci. 8 (1613).

John, R., Anjum, N.A., Sopory, S.K., Akram, N.A., Ashraf, M., 2016. Some key physiological and molecular processes of cold acclimation. Biol. Plant 4, 603–618.

Kant, S., Burch, D., Badenhorst, P., Palanisamy, R., Mason, J., Spangenberg, G., 2015. Regulated expression of a cytokinin biosynthesis gene IPT delays leaf senescence and improves yield under rainfed and irrigated conditions in canola (*Brassica napus* L.). PLoS One 10, e0116349.

Khodakovskaya, M., McAvoy, R., Peters, J., Wu, H., Li, Y., 2006. Enhanced cold tolerance in transgenic tomato expressing a chloroplast ω-3 fatty acid desaturase gene under the control of a cold-inducible promoter. Planta 223, 1090–1100.

Kirst, H., Garcia-Cerdan, J.G., Zurbriggen, A., Ruehle, T., Melis, A., 2012. Truncated photosystem chlorophyll antenna size in the green microalga *Chlamydomonas reinhardtii* upon deletion of the *TLA3-CpSRP43* gene. Plant Physiol. 160, 2251–2260.

Levitt, J., 1980. Responses of Plants to Environmental Stresses. Academic Press, New York.

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.

Ma, X., Zhang, J., Burgess, P., Rossi, S., Huang, B., 2018. Interactive effects of melatonin and cytokinin on alleviating drought-induced leaf senescence in creeping bentgrass (*Agrostis stolonifera*). Environ. Exp. Bot. 45, 1–11.

Megha, S., Basu, U., Kav, N.V., 2018. Regulation of low temperature stress in plants by microRNAs. Plant Cell Environ. 41, 1–15.

Müller, M., Munné-Bosch, S., 2011. Rapid and sensitive hormonal profiling of complex plant samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Plant Meth 7 (37).

Preston, J.C., Sandve, S.R., 2013. Adaptation to seasonality and the winter freeze. Front. Plant Sci. 4 (167).

Reiter, R.J., Tan, D.X., Manchester, L.C., Qi, W., 2001. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. Cell Biochem. Biophys. 34, 237–256.

Sanghera, G.S., Wani, S.H., Hussain, W., Singh, N., 2011. Engineering cold stress tolerance in crop plants. Curr Genom. 12, 30–43.

Scott, I.M., Clarke, S.M., Wood, J.E., Mur, L.A.J., 2004. Salicylate accumulation inhibits growth at chilling temperature in Arabidopsis. Plant Physiol. 135, 1040–1049.

Soliman, M.H., Alayafi, A.A.M., El Kelish, A.A.E., Abu-Elsaoud, A.M., 2018. Acetylsalicylic acid enhance tolerance of *Phaseolus vulgaris* L. To chilling stress, improving photosynthesis, antioxidants and expression of cold stress responsive genes. Bot. Stud. 59 (6).

Tan, D.-X., Hardeland, R., Manchester, L.C., Korkmaz, A., Ma, S., Rosales-Corral, S., Reiter, R.J., 2012. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. J. Exp. Bot. 63, 577–597.

Tan, D.-X., Manchester, L.C., Liu, X., Rosales-Corral, S.A., Acuna-Castroviejo, D., Reiter, R.J., 2013. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. J. Pineal Res. 54, 127–138.

Zhang, H., Sonnewald, U., 2017. Differences and commonalities of plant responses to single and combined stresses. Plant J. 90, 839–855.

Zhang, N., Sun, Q., Zhang, H., CaoY, Weeda S., Ren, S., Guo, Y.-D., 2014. Role of melatonin in abiotic stress resistance in plants. J. Exp. Bot. 66, 647–656.

Zhao, H., Zhang, K., Zhou, X., Xi, L., Wang, Y., Xu, H., Pan, T., Zou, Z., 2017. Melatonin alleviates chilling stress in cucumber seedlings by up-regulation of *CsZat12* and modulation of polyamine and abscisic acid metabolism. Sci. Rep. 7, 4998.

Capítol 5. La major tolerància de l'espècie invasora *Carpobrotus edulis* a l'estrès per baixes temperatures ha permès la seva l'expansió a nous territoris

Chapter 5. Increased chilling tolerance of the invasive species Carpobrotus edulis may explain its expansion across new territories

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Research article

Increased chilling tolerance of the invasive species *Carpobrotus edulis* may explain its expansion across new territories

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[†]E.F. and S.M.B. conceived the ideas and designed the methodology. E.F. collected and analysed the data and wrote the manuscript. S.M.B. revised the manuscript provided research tools and reagents, and supervised the project.

Invasive plants are expanding their geographical distribution across new regions. Expansion modeling is crucial for geographic prioritization in management policies. However, the assumption of niche conservatism and the lack of information of the species physiological response to the environmental factors determining species presence may hinder predictions. In this study, we aimed to understand the expansion of the widely distributed plant *Carpobrotus edulis* in Europe. We contrasted introduced and native *C. edulis* ecological niches and explored the experimental response to temperature, a major determining factor for species distribution, of native and invasive individuals in terms of different biochemical markers. Niche analysis revealed an expansion of the introduced niche to occupy colder climates. Introduced and native individuals showed differential mechanisms facing low temperatures. Individuals from the native range showed an increased sensitivity to chilling, as reflected by photosynthetic pigment degradation, increased de-epoxidation of xanthophylls and the accumulation of the lipophilic antioxidant alpha-tocopherol. The found physiological differentiation towards an increased invasive chilling tolerance of invasive *C. edulis* individuals together with a high propagule pressure may explain the introduced climatic niche shift to colder climates observed, allowing the extensive expansion of this species in Europe.

Key words: Aizoaceae, antioxidants, performance, photoprotection, random forest, species distribution model

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Introduction

Despite invasive species constituting one of the main threats to global biodiversity, the key factors determining their success when colonizing extensive areas are still unknown (Simberloff *et al.*, 2013). No single factor explains the species distribution limits and their expansion, but propagule pressure, environmental suitability and biotic relationships may have a role during species expansion across new territories out of their native geographical distribution (Willi and Van, 2019). Besides those factors, the study of invasive species expansion reinforced the importance of rapid evolutionary changes determining the invasive success (Bossdorf *et al.*, 2005). In the introduced range, the evolution of functional traits or plasticity might occur as an adaptive response to novel biotic and abiotic environmental pressures. Three light intensities were used to contrast native and invasive populations of *Chromolaena odorata* revealing contrasted phenotypic plasticity between species origin (Liao *et al*., 2019). The comparison of several functional traits of *Acer pseudoplatanus* growing in France (native) and New Zealand (invaded range) under different light regimes revealed also higher plasticity and faster growth of invasive individuals (Shouman *et al*., 2017). Increased biotic tolerance was found to be genetically determined in *Lythrum salicaria* invasive populations, compared to native individuals grown under the same environmental conditions (Joshi and Tielbörger, 2012). Despite genetically contrasted populations between species' native and introduced ranges, niche conservatism between species' ranges is one of the assumptions of species distribution modeling (SDM), which constitutes a promising tool to predict suitable areas for the establishment of alien species and the likelihood of the risk of invasion (Peterson, 2003; Thuiller *et al*., 2005; Kearney and Porter, 2009). Moreover, SDM also assumes that actual introduced and native range occurrences define the species' response to environmental variables (Peterson, 2003), with no clue as to the species' potential to respond to environmental conditions.

Temperature is one of the main environmental variables limiting plant growth and is therefore a major determining factor in the distribution of plants across different environments (Mittler, 2006). In the case of the invasive species *Amaranthus palmeri,* mean annual temperature has been identified to limit its northward range expansion (Briscoe Runquist *et al*., 2019). Plant response to temperature is complex as several mechanisms at different levels interact to give differential responses accordingly with the magnitude of the temperature change. In fact, cold tolerance is thought to be a difficult trait to acquire, as most angiosperms evolved in tropical climates (Preston and Sandve, 2013). Plant responses may be different in response to temperatures from 15 to 0°C (chilling) from temperatures below water melting point (freezing), which may involve ice formation (Levitt, 1980). Plants experience chilling as part of seasonal events in cold temperate zones and tropical highlands. Plant response to chilling involves alterations in water balance, the suppression of the main metabolic pathways, and an increase of various protective molecules (antioxidants, biomolecule stabilizers and osmoregulators) (John *et al*., 2016). Furthermore, stress by cold conditions is described to lead to reactive oxygen species (ROS) overproduction, capable of severely damaging all the biomolecules and even cause cell death (Suzuki and Mittler, 2006). The synthesis of antioxidants and photoprotective compounds may be a part of a complex reaction mechanism to low-temperature stress involving both protection against and avoidance of ROS production (Cansev *et al*., 2012) and can therefore be used to determine plant sensitivity to cold. A higher capacity to survive and reproduce at different temperatures through this physiological and biochemical adaptations may determine the persistence of a species in a specific location, and could therefore be explaining invasive species success and expansion.

Carpobrotus edulis (L.) N.E. Br. (Aizoaceae) is a succulent mat-forming clonal plant native to the Cape Region in South Africa. Despite its restricted native distribution, this species has colonized different coastal Mediterranean regions around the world, becoming an aggressive invader (D'Antonio and Mahall, 1991; Vilà *et al*., 2008). *C. edulis* have shown a great expansion around the Mediterranean Sea, being found in almost all countries of the west Europe, where some of them (Spain, Portugal, France, Italy, Gibraltar and Azores) are considering this species a high threat to their biodiversity (Campoy *et al*., 2018). Clonal propagation has been proposed as a key trait in determining this species' success (Roiloa *et al*., 2010, 2013, 2014a, 2014b), allowing it to sustain growth in hostile habitats making them habitable after successive death cycles (Fenollosa *et al*., 2016). Moreover, allelopathic compounds released by the species inhibit native species germination, which contributes to the competitive abilities of *C. edulis* (Conser and Connor, 2009; Novoa and González, 2014). Although these traits may contribute to the increased vigor of *C. edulis*¸ few studies have been conducted exploring the differentiation between individuals from native and invasive ranges (Roiloa *et al*., 2016), and none has studied the existence of a differential response to temperature between invasive and native individuals which may be determinant in explaining this species' expansion.

In the present study, we aimed to understand the *C. edulis* expansion in Europe through an ecophysiological approach. We contrasted introduced and native *C. edulis* ecological niche and explored physiological intraspecific variability of the experimental response to temperature of individuals from both ranges. We explored different physiological responses to low temperatures in terms of different cold-sensitive biochemical markers regarding water balance, photosynthetic efficiency and the content and composition of photoprotective molecules between native and invasive individuals of *C. edulis*. We hypothesized that (i) introduced and native *C. edulis* niches may be different, (ii) individuals from the native and invasive ranges respond differently to temperature, (iii) *C. edulis* has physiological mechanisms to withstand chilling and (iv) species expansion has occurred thanks to a differential physiological response of the introduced and native ranges. First, we analysed the climatic niche of the species to describe the niche dynamics resulting from the invasion process. Secondly, we monitored the response of *C. edulis* to temperature in both an experimental garden and controlled conditions, using individuals from the native (South Africa) and the invasive (Spain) ranges. The combination of the species' response to a main distribution variable with niche modeling helps us understanding the expansion of *C. edulis*.

Material and methods

Climatic niche analysis

To describe the species' climatic niche and evaluate the existence of differential climatic niches between introduced and native ranges (hypothesis 1), we conducted environmental niche modeling based on the species' occurrences in its native and European introduced ranges. We used the bioclimatic variables from the WorldClim 2 database at 0.5 arcmin resolution (~1 km^2) (Fick and Hijmans, 2017), and available presence-only data for *C. edulis* in the Global Biodiversity Information Facility (GBIF) database (http://www.gbif.org, accessed in March 2018 using the 'dismo' R package). Occurrences were individually inspected to ensure credibility and geospatial accuracy. Moreover, we removed duplicate occurrences (within 1 km of each other). After filtering, 544 records remained, 492 from Europe and 52 from the native range in South Africa. Climatic niche overlap was quantified on the multivariate environmental space derived from a principal component analysis (PCA) using the WorldClim 2 bioclimatic variables. The entire environmental space of the two studied areas was used to calibrate the PCA and resulted to the PCA-env that was used for the analysis (Broennimann *et al.*, 2012). A kernel density function was applied to smooth the occurrences density obtained from the PCA-env, minimizing sampling bias (Warren *et al.*, 2008). The Schoener's D metric was used to estimate niche overlap contrasting the two ranges occupancy of the same climatic space ranging from 0 (no overlap at all) to 1 (complete overlapping) (Schoener, 1970). To test differences between range's climatic niches, two different tests were used using the niche overlap values: niche equivalence and similarity. Niche equivalence explores the consistence of the niche overlap when randomly reallocating occurrences from both ranges maintaining constant the observed frequencies. Niche similarity addresses whether the environmental niche occupied in one range is more similar to the one occupied in the other range than would be expected by chance. After 1000 iterations, a histogram of simulated values is obtained in both cases and niche equivalency or similarity is rejected if the observed D falls within the 5% density of simulated values.

In order to describe the potential distribution (i.e. the predicted area where the species could be found based on the projection of the climatic niche over the territory) of *C. edulis*, a SDM was build using species occurrences and WorldClim 2 bioclimatic variables. An only-presence maximum entropy model (MaxEnt) was used (Phillips *et al.*, 2006, 2018), with 5*k*-fold cross validation. To link the experimental results with the model, we contrast the native SDM (using the occurrences from the native range) with the European SDM (using the occurrences from Europe). Suitability change between SDMs was obtained as raster difference between models. A final mean value of suitability difference in Europe was calculated as:

$$\text{Increased introduced suitability} = \frac{\sum_{i,j}(\delta_{\text{European}} - \delta_{\text{Native}})}{\sum_{i,j}(\delta_{\text{European}})} * 100$$

where δ stands for model predicted suitability. Increased introduced suitability was calculated considering all *k*-fold combination of native and European SDMs, thus obtaining a variability measure.

Experimental response to cold temperature of native and invasive individuals

Two experiments to evaluate physiological intraspecific variability species response to winter in the invasive range (Experiment 1) and to evaluate species cold response under controlled conditions (Experiment 2) were performed with native and invasive individuals of *C. edulis* (hypothesis 2 and 3). The realization of both experiments allowed us to confirm the obtained results during natural annual cold (winter) and when temperature is isolated. Seeds from the native and invasive ranges of *C. edulis* were collected in the region of Fish Hoek in South Africa (34°07′S, 18°25′E) and in a protected area in the Cap de Creus Natural Park in Spain (42°21′N, 3°11′E), respectively. About 10 dehydrated fruits from 10 non-connected *C. edulis* clumps at least 10 m apart were collected and stored at 4°C until germination. After seed disinfection with commercial bleach, pooled seeds were germinated under a light cycle of 12:12 h (light:dark hours), at 21°C and 65% humidity. After 4 months, *C. edulis* individuals were transferred into 1 L pots in the greenhouse and morphologically similar individuals from both ranges were placed in the experimental garden of the Faculty of Biology (Barcelona, NE, Spain) under Mediterranean winter conditions of the introduced range to perform Experiment 1. The other individuals were kept in the greenhouse until the chambers were ready for the experiment under controlled conditions (Experiment 2), which was performed in May 2017.

Experiment 1 was designed to monitor the response of *C. edulis* to the low temperatures in its introduced range. At the experimental garden, six individuals per range were sampled at five different points in time from December 2016 (first sampling on 29 December) to February 2017. During January, a cold spell was forecasted and the plants were sampled just before (second sampling point – 13 January) during (third sampling point – 17 January), just after it (fourth sampling point – 24 January) and 1 month after (fifth sampling point – 27 February). During the cold spell, the minimum temperature registered was 1°C. All selected days were clear days and sampling was performed at solar midday to guarantee comparable photoprotective responses. The meteorological conditions registered by the nearest automatic meteorological station (located at 1.4 km) are described in Table 1. Plants were watered two to three times per week according to evapotranspiration demand to ensure water content to reach field capacity. Experiment 2 evaluated the response to low temperatures under controlled conditions, using two identically controlled chambers (Ibercex model E-1350-DV, Madrid, Spain) (light cycle of 12:12 h light:dark). About 20 morphologically similar individuals of *C. edulis* from each range were transferred to the chambers after 3 months of light acclimation. One chamber temperature was kept at ~21°C, whereas the other was set at 8.6°C after 7 days at 14°C (Table 1). Field capacity was ensured by watering plants two times per week. After 7 days at 8.6°C, sampling was

Table 1: Meteorological conditions for Experiment 1 (experimental garden) and Experiment 2 (controlled conditions). Bold values show samplings with lowest temperatures

	Experiment 1					Experiment 2	
	Dec 29	Jan 13	Jan 17	Jan 24	Feb 27	Control	Cold
Min. temp. (°C)	4.6	**4.7**	**1.0**	**5.8**	9.6	20.5	**7.5**
Mean temp. (°C)	10.3	**10.6**	**4.7**	**8.6**	12.6	21	**8.6**
Max. temp. (°C)	16.4	**13.7**	**7.8**	**14.5**	16.2	23.5	**10**
Max. VPD (mbar)	12.8	**10.4**	**8.4**	**8.5**	6.8	10.7	**4.2**
Max. global solar radiation (W m^{-2})	452	**481**	**497**	**628**	654	181	**181**

performed simultaneously in both chambers at the middle of the photoperiod. The number of replicates was $n = 5$.

Three leaves per plant were taken at midday on sampling days. One was used for assessment of leaf water content and chlorophyll fluorescence measurements, and two were immediately frozen in liquid nitrogen and stored at −80°C until biochemical analysis of photoprotective compounds and antioxidants that describe plant sensitivity to stress by cold conditions. Moreover, stomatal conductance (gs) was measured at solar midday in situ in three young but fully-developed leaves per plant using a leaf porometer (SC-1 Leaf Porometer; Meter Group, Pullman, USA). Light conditions during sampling correspond to the daily maximum global solar radiation described in Table 1 for each sampling day. Leaf water content (H) was calculated as (FW – DW)/DW, where FW is fresh weight and DW dry weight, determined at 60°C at constant weight. To estimate photosynthetic efficiency, chlorophyll fluorescence parameters were determined by the saturation pulse method using a portable pulse amplitude-modulate fluorometer (MINI-PAM photosynthesis yield analyser; Walz, Effeltrich, Germany) on fully developed and illuminated leaves. The relative efficiency of photosystem II (φ_{PSII}) was measured under incident irradiation. The maximum quantum yield of photosystem II (F_v/F_m) was measured after a 30-min dark adaptation period. Photosynthetic pigments (chlorophylls *a* and *b*, chlorophyll *a*/*b* ratio, zeaxanthin, antheraxanthin, violaxanthin, lutein and β-carotene), and α-tocopherol (the main form of vitamin E) were measured as stress markers. The de-epoxidation state (DPS) of the xanthophylls was calculated as (Zx + Ax)/VAZ, where Zx stands for zeaxanthin, Ax for anteraxanthin and VAZ for the sum of violaxanthin, anteraxanthin and zeaxanthin. Pigment content and composition are both good stress markers that reflect plant stress sensitivity and are commonly used in physiological studies (Pintó-Marijuan and Munné–Bosch, 2014). Leaf samples were ground in liquid nitrogen using a mix ball and extracted with cold methanol containing 0.01% butylated hydroxyltoluene using ultrasonication. After centrifuging at 12 000 rpm for 10 min at 4°C, the supernatant was collected and the pellet re-extracted with the same solvent until it was colourless; then, supernatants were pooled and filtered with 0.22 μm and transferred to high-performance liquid chromatography (HPLC) vials. Photosynthetic pigments were separated on a binary-solvent gradient using a reverse-phase HPLC system and quantified with a diode array detector, as described by Munné-Bosch and Alegre (2000). Alpha tocopherol was separated isocratically in a normal-phase HPLC system and quantified with a fluorescent detector, as described by Amaral *et al*. (2005). Quantification was based on the results obtained from the fluorescence signal and compared to that of a calibration curve made with an authentic standard (Sigma-Aldrich, Steinheim, Germany).

Linking physiology and ecological performance

To understand the link between the distribution of *C. edulis* and the species physiological response to temperature and unravel if differential physiological responses determine species expansion (hypothesis 4), Classification and Regression Random Forest analysis were performed to capture non-linear multivariant responses. Random Forest (RF) is a flexible machine learning algorithm that can be used both for regression and classification. Random Forest Classification was performed to capture the physiological differentiation between ranges and Random Forest Regression to predict species distribution. We hypothesized that the relative abundancy of *C. edulis* at different temperatures in Europe may be explained by the observed physiological values in the performed experiments. To contrast that hypothesis, we obtained relative abundancy values from GBIF occurrences (from now on: Performance) linked to a temperature variable. We selected mean temperature of the coldest quarter of the year (BIO11), as it better represents the mean temperature evaluated period in Experiment 1, performed on the coldest quarter of the year. Data from controlled conditions were not used for this analysis, as light conditions were not comparable.

Statistics

Niche analysis was performed with the *ecospat* package (Broennimann *et al*., 2016). SDM was performed using the packages *dismo* and *rmaxent*. A two-way mixed ANOVA of repeated measures with 'Range' as a between-samples factor and 'Time' as a within-samples factor was performed for Experiment 1. A two-way ANOVA with 'Range' and 'Treatment' was performed for Experiment 2. The Tuckey test was used as a *post-hoc* method. Data were tested with Shapiro-Wilk and Levene tests for normality and homocedasticity and transformed whenever necessary. All analyses were performed using the *nlme* and *multcomp* packages in R 3.3.3. Random Forest analysis was performed using *randomForestSRC* and *ggRandomForests* packages, and the workflow suggested in Feld *et al.* (2016).

Results

Differential climatic niches between origins

Niche analysis revealed a broader introduced niche, expanded to lower temperatures (Fig. 1), supporting our first hypothesis. Considering the European and native ranges, the distribution of *C. edulis* is constrained to annual mean temperatures of between 7 and 20°C and annual precipitation ranging from 100 to 1500 mm (Fig. 1A). PCA-env analysis found two main components, which represent mean coldness and precipitation (PC1) and maximum coldness and seasonality (PC2), as revealed by the weights of the variables (Fig. 1B). The comparison of the niche area plotted onto the multivariate space by the native and the European ranges revealed a broader niche considering the introduced range towards low temperatures (Fig. 1C). Little overlap was found between native and introduced ranges, as Schoener's $D = 0.373$, meaning that niche overlap between native and introduced ranges is lower than 40%. Niche similarity and equivalence were discarded (P-value < 0.05 in both cases) (Fig. 1C), revealing that niches are not equivalent in the different geographical areas and that the introduced niche tends to be more similar to random than native niche.

The designed SDM for the native and the European *C. edulis* resulted in completely different introduced projections on Europe (Fig. 2). The suitability (0–1) values obtained when projecting the native niche over Europe were extremely low in comparison with the introduced projection. The comparison of both projections revealed a $94.52\% \pm 0.15$ difference at the introduced range. In other words, the niche difference between introduced and native ranges of *C. edulis* leads to extremely different (more than 90%) potentially habitable areas in Europe (Fig. 2).

Physiological response to chilling

Chilling induced alterations in *C. edulis* water balance (Fig. 3A–D). With the arrival of the cold spell, a significant decay was detected in hydration and stomatal conductance. The lowest values of leaf hydration (H) were registered at the lowest air temperatures (7.8°C during sampling). In spite of the fact that hydration remained low during the cold spell, transpiration was restricted only at the beginning. The lowest registered stomatal conductance was 61.73 ± 8.18 mmol m^{-2} s^{-1}, which represented a reduction of ~60% from the highest mean value observed (air temperature = 15.6°C). Despite different vapor pressure deficit (VPD) between experiments (Table 1), similar leaf hydration was found in the control individuals under controlled conditions in comparison to the results at the experimental garden, with equivalent reductions when the temperature fell below 10°C (Fig. 3A and B). Despite non-significant differences between treatments, lower values of stomatal conductance were registered for the cold treatments (Fig. 3D). No significant differences ($P > 0.05$) in any of the tested conditions were found between ranges for hydration and stomatal conductance.

C. edulis individuals from the native and invasive ranges behaved differently in response to chilling regarding photoprotective responses (Figs 3–5). Despite common alterations being observed in both native and invasive *C. edulis*, they occurred at different chilling intensities. Individuals from both ranges showed a significant decrease (~42%) and recovery in the relative efficiency of photosystem II (Φ_{PSII}) in response to low temperatures in Experiment 1 (Fig. 3E). No alterations in relative photosystem II efficiency were detected when contrasting both ranges under controlled conditions, under lower irradiation (Table 1). In spite of the fact that no photoinhibition was observed (all registered values of the maximum yield of photosystem II, i.e. F_v/F_m, were above 0.75, Fig. 3G and H), the cold spell induced range differentiation. *C. edulis* from the native range recorded lower F_v/F_m values at the end of the cold spell (Fig. 3G).

Chilling induced changes not only in the photochemical yield but also in the photosynthetic pigments composition and lipophilic antioxidant system (Figs 4 and 5). Both ranges responded to low temperatures with a reduction of chlorophylls, and an increased the proportion of chlorophyll *a* to *b* (i.e. Chl *a*/*b*), the DPS of the xanthophylls and zeaxanthin content. However, stress indicators such as the chlorophyll content, Chl *a*/*b*, the xanthophyll pool (VAZ) and its DPS of the two compared ranges of *C. edulis* responded at different chilling intensities leading to significant differences between ranges (Figs 4 and 5A). The DPS increased below 10°C as in Experiment 1 (Fig. 4E and F). The significant zeaxanthin (Zx) content increase in individuals from the native range under cold conditions in Experiment 2 (Fig. 5D) is responsible of the greater DPS increase in the native individuals under cold conditions, in comparison to the invasive individuals (Fig. 4F). Higher contents of lutein (Lut) were found in the native individuals in Experiment 2 (Fig. 5F) despite similar values of this xanthophyll being found in Experiment 1 (Fig. 5E). Despite no significant differences between ranges were found for β-carotene (β-Car) in Experiment 1, mean values at the first sampling point were similar to the values found at 21°C in Experiment 2, where native individuals showed significantly higher β-Car content (Fig. 5G and H). Alpha-tocopherol was found to increase significantly in the

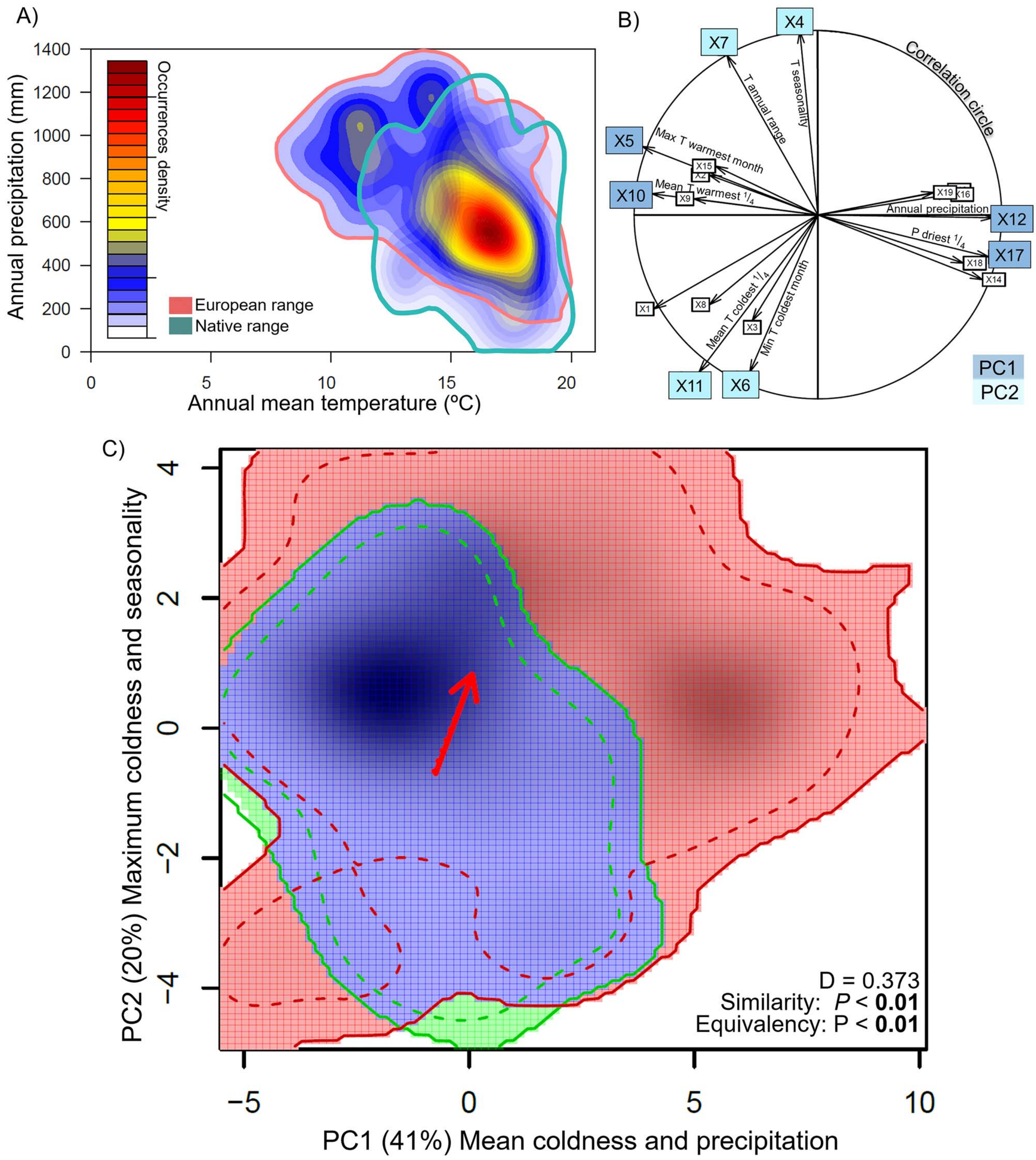

Figure 1: (A) Kernel density estimation for *C. edulis* occurrences in response to annual mean temperature and precipitation. (B) Correlation circle for the PCA-env analysis, with the 19 bioclimatic WorldClim variables (X1-19). Bioclimatic variables full names can be found at: http://worldclim.org/bioclim. (C) Niche dynamics: stability, expansion and unfilling (in blue, red and green respectively) in the multivariate climatic space for native compared to the European niche of *C. edulis* considering the two first components from the PCA-env. D Stands for Schoener's D overlap value. Solid and dashed lines delineate 100 and 75% of the available background environment, respectively.

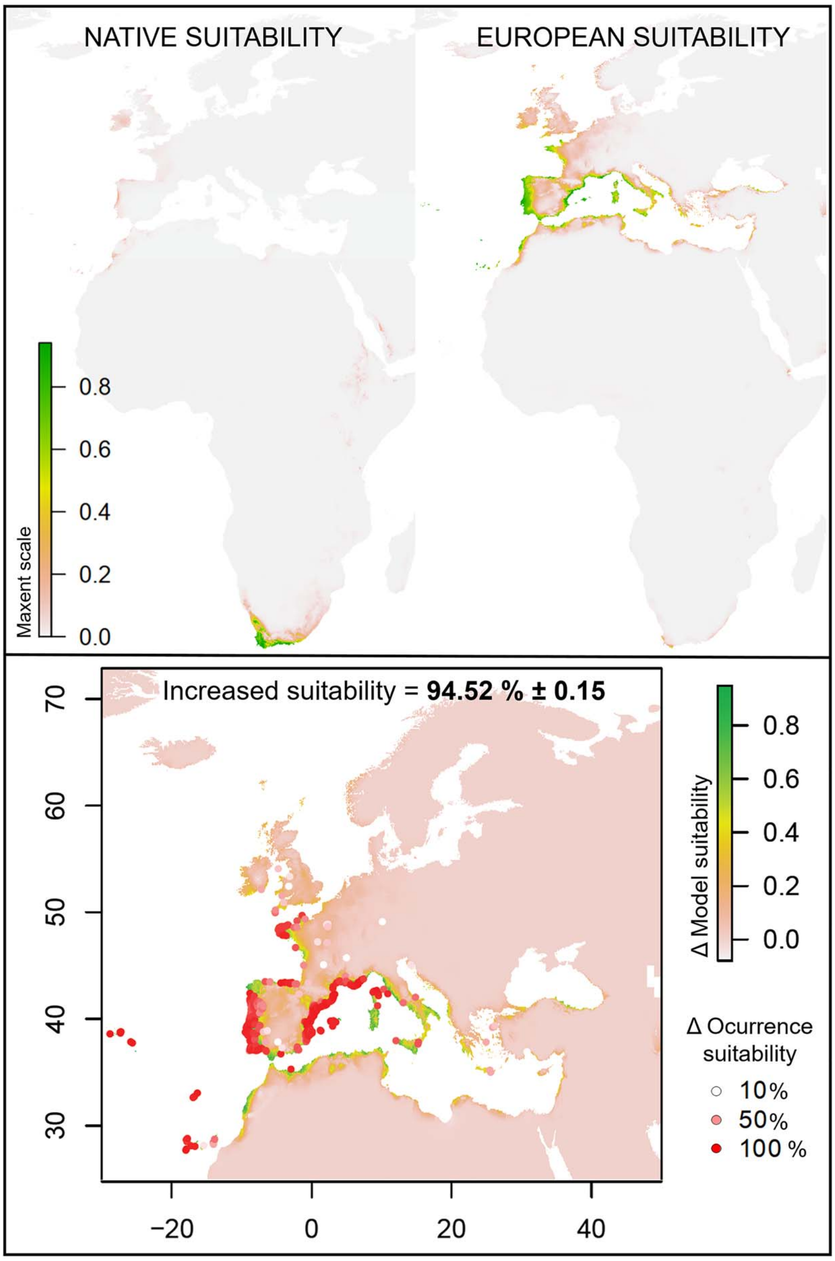

Figure 2: MaxEnt predicted suitability considering the native and the European occurrences of *C. edulis* (Native and European suitability, respectively) and difference between both projections (Increased suitability). Dots correspond to actual invasive occurrences, coloured by the percentage of increased suitability between native and European projections.

native but not in the invasive individuals of *C. edulis* with the arrival of the cold spell (Fig. 5I). Similarly, under controlled conditions, individuals from the native range had a higher α-toc content when temperatures fell below 10°C in comparison to α-Toc content of the invasive individuals (Fig. 5J).

Differential chilling responses determine species performance

The different relative density of occurrences of *C. edulis* under different values of mean temperature of the coldest quarter (BIO11) differed between species ranges, especially under low temperatures between 0 and 5°C, where the native range does not show any occurrence (Fig. 6A.2). The European range showed higher performance in all of the experimental temperatures except for 12.6°C (Fig. 6A.3). On one hand, the obtained Classification Random Forest algorithm revealed differential variable importance for the two ranges (Fig. 6A.1). On the other hand, the obtained Regression Random Forest revealed that 27.3 and 61.7% of the introduced and native performance variability, respectively, was explained by physiological variables (Fig. 6B). The important

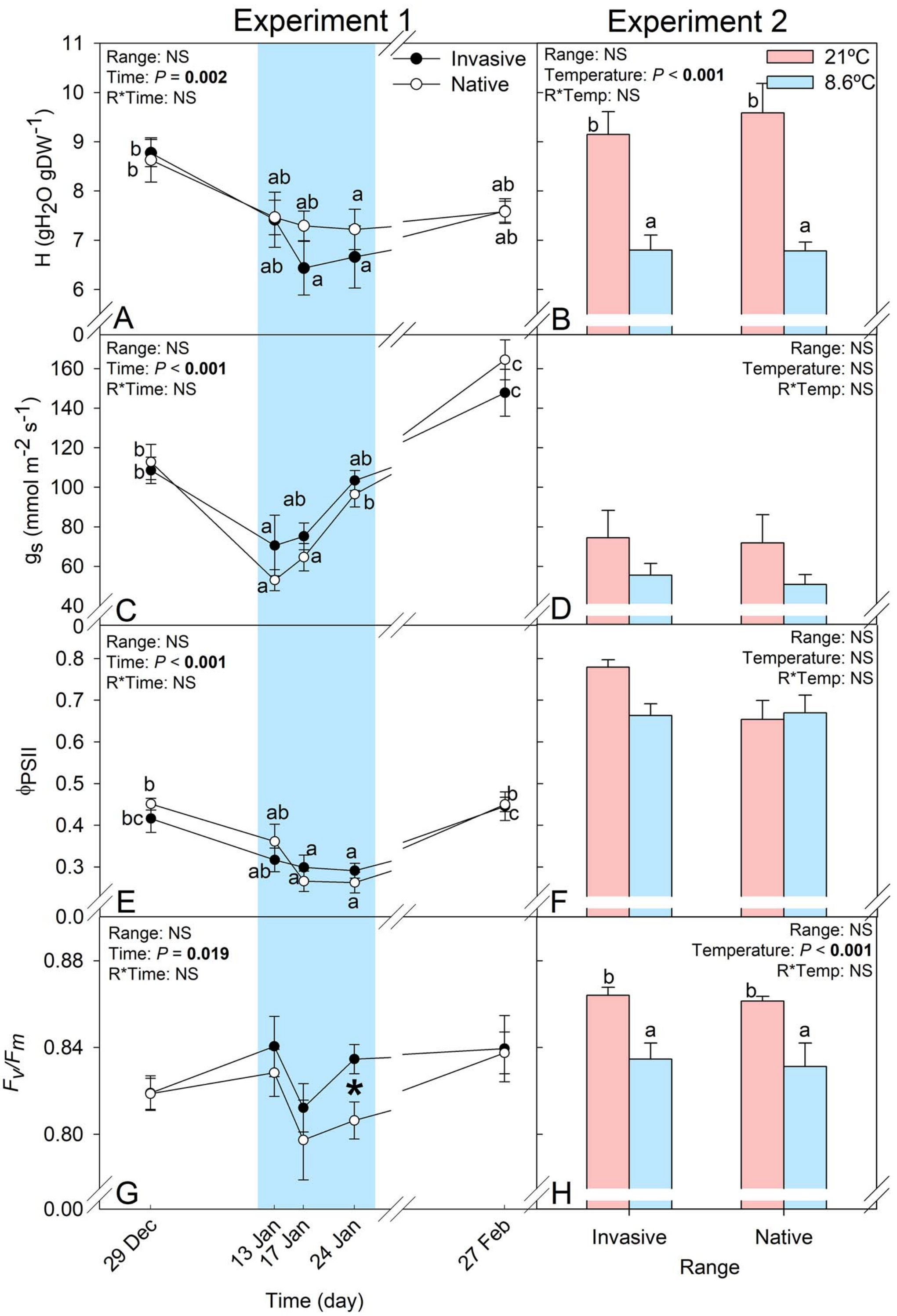

Figure 3: Leaf hydration (H), stomatal conductance (g_s), maximum (F_v/F_m) and relative (φ_{PSII}) efficiency of photosystem II of individuals of *C. edulis* collected in its native (South Africa) and invasive (Spain) ranges in Experiment 1 at experimental garden and 2 under controlled conditions. Blue highlights samplings with lowest temperatures. In the left panels, letters represent significant differences ($P < 0.05$) between sampling dates within each range, whereas in the right panel, represent significant differences between the different bars. Data are shown as mean ± standard error (SE).

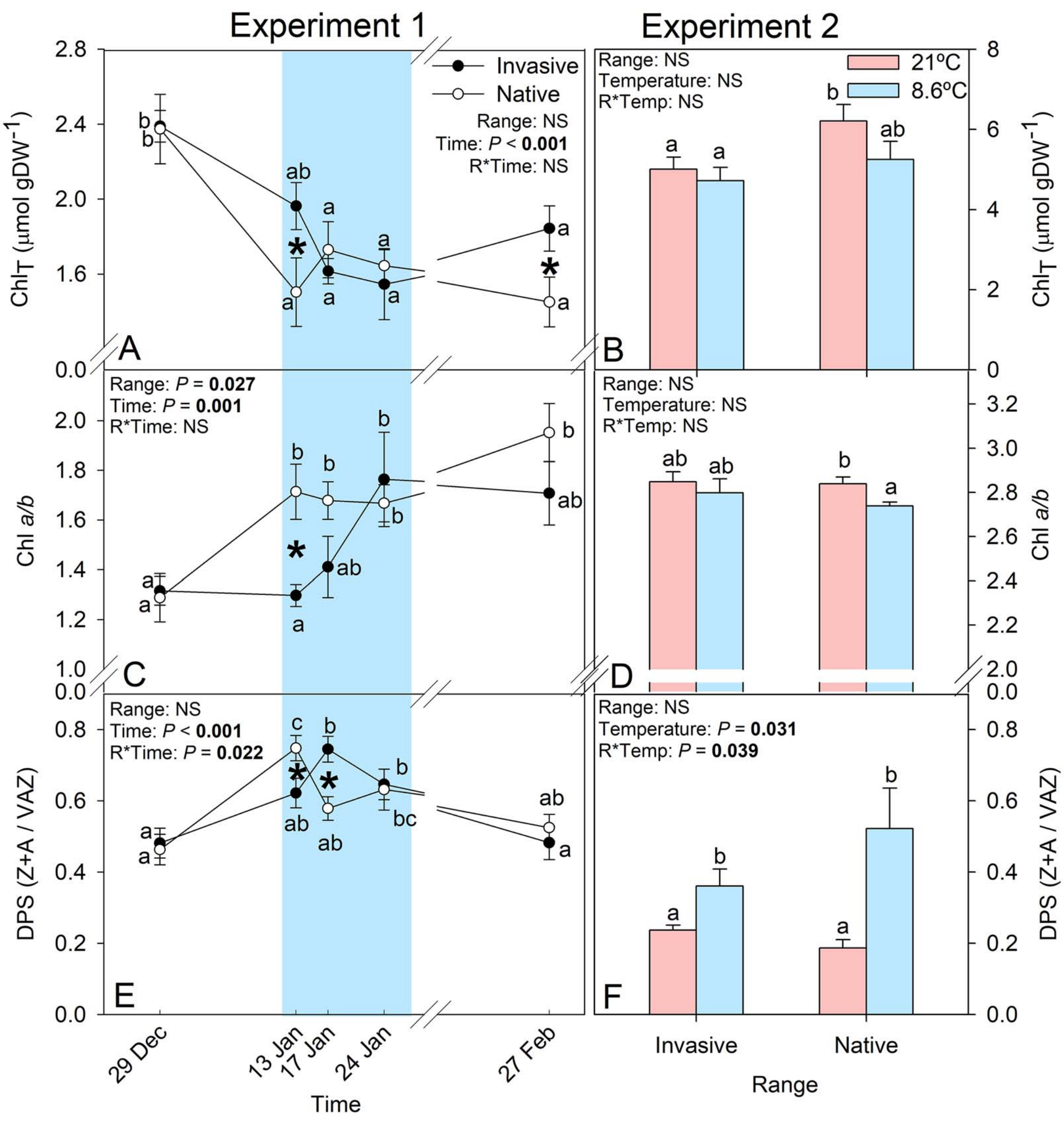

Figure 4: Total chlorophylls ($\mathrm{Chl_T}$), chlorophyll *a*/*b* ratio and xanthophyll DPS of individuals of *C. edulis* collected in its native (South Africa) and invasive (Spain) ranges in Experiment 1 at experimental garden and 2 under controlled conditions. Blue highlights samplings with lowest temperatures. In the left panels, letters represent significant differences ($P < 0.05$) between sampling dates within each range, whereas in the right panel, represent significant differences between the different bars. Data are shown as mean ± SE.

variables for each model were different, with the exception of Chl *a*/*b* ratio. Some clear non-linear responses were observed for some variables. VAZ, $\mathrm{Chl_T}$, β-Car, α-toc and H positively contributed to increased performance in the invasive individuals. For the native individuals, ΦPSII, gs, Chl *a*/*b* and F_v/F_m positively contributed to performance, whereas Zx and VAZ/$\mathrm{Chl_T}$ contributed negatively.

Discussion

Temperature is one of the main environmental variables that define plant species' distribution, as it constitutes one of the main constrains to plant growth and development, causing stress, damage and even plant death (Mittler, 2006). In our study, the physiology of *C. edulis* was affected by chilling with alterations at multiple levels. The differential chilling sensitivity between ranges exposes the great capacity of this species to adapt to different conditions, allowing this plant to expand its geographical distribution. Photosynthetic efficiency, photosystem composition and antioxidant response showed a consistent pattern of increased sensitivity by the individuals from the native range of *C. edulis*.

The chilling response of *C. edulis* was characterized by water loss, a fall in the relative efficiency of photosystem II, degradation of chloroplast pigments and an increase in the de-epooxidation state of the xanthophyll cycle. Stomatal closure unbalanced redox potential due to photosynthetic substrate limitation and limited photosynthetic efficiency. Individuals

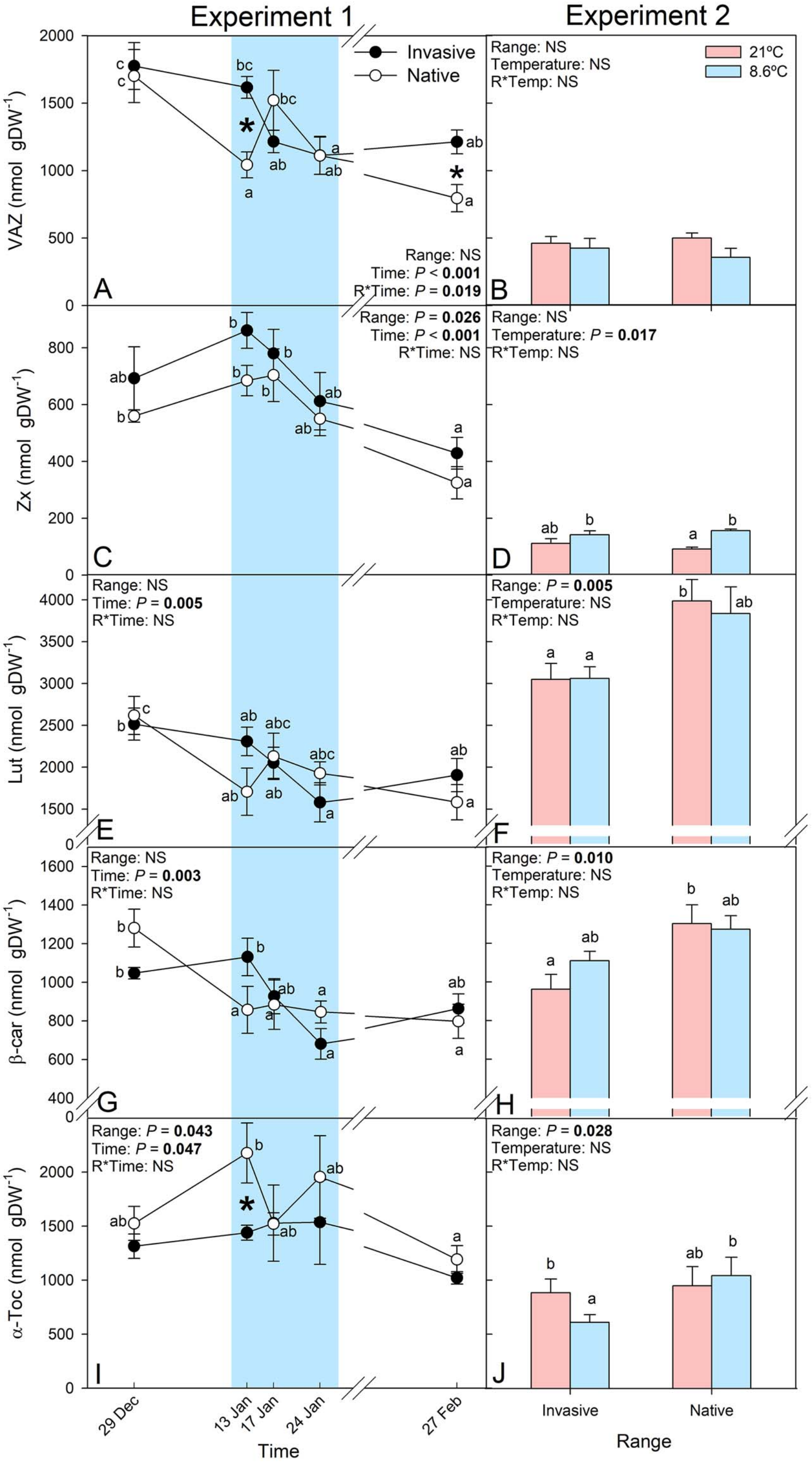

Figure 5: Photoprotective compounds content. Total xanthophyll pool (VAZ), zeaxanthin (Zx), lutein (Lut), β-carotene (β-car) and α-tocopherol (α-Toc) of individuals of *C. edulis* collected in its native (South Africa) and invasive (Spain) ranges in Experiment 1 at experimental garden and 2 under controlled conditions. Blue highlights samplings with lowest temperatures. In the left panels, letters represent significant differences ($P < 0.05$) between sampling dates within each range, whereas in the right panel, represent significant differences between the different bars. Data are shown as mean ± SE.

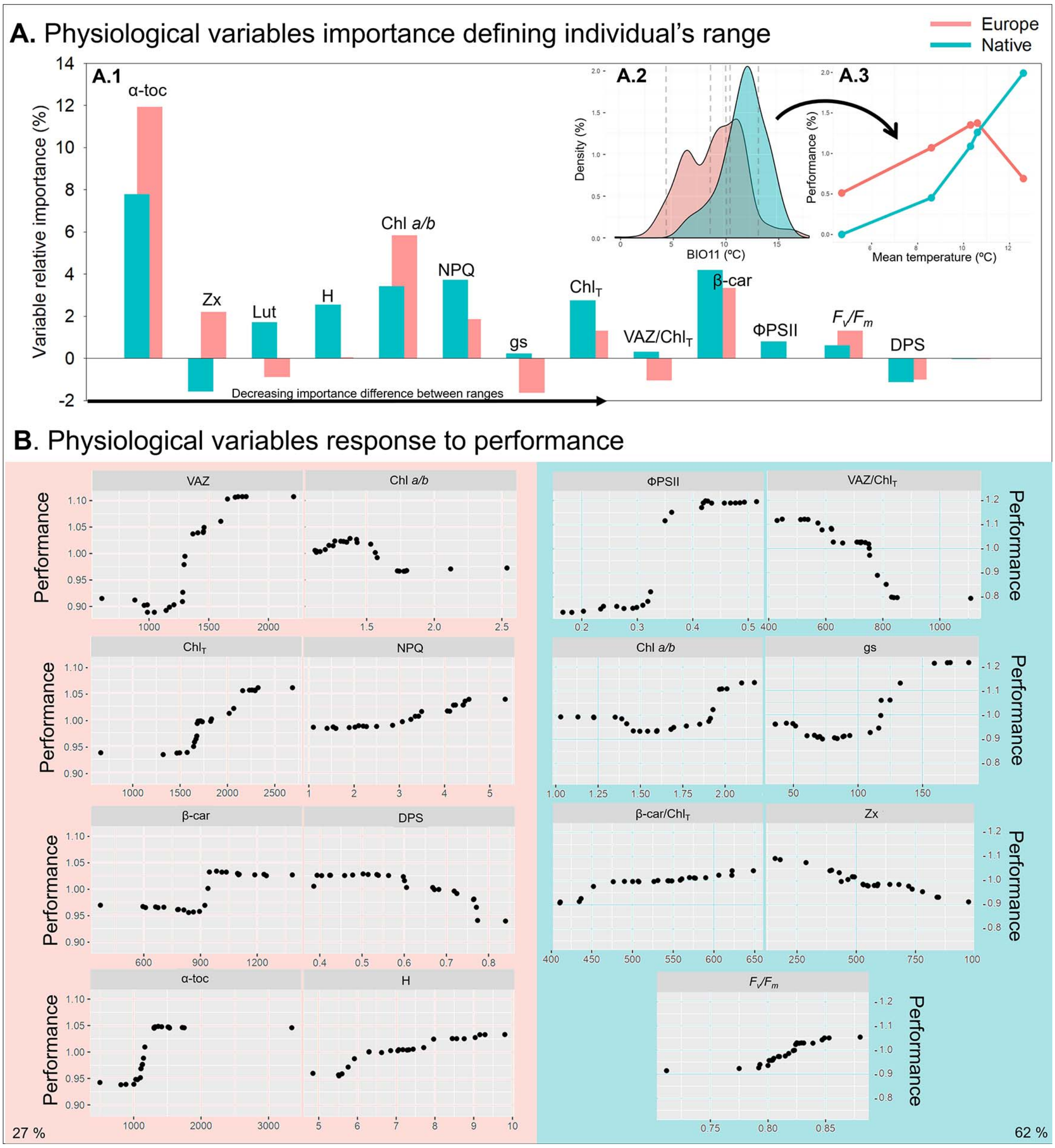

Figure 6: Random Forest classification (A) and regression (B) analysis results for "range" and "performance" prediction respectively. (A.1) Variables relative importance for the European and native models at the classification RF analysis. (A.2) Density of occurrences at different values of mean temperature of the coldest quarter of the year (BIO11) of the European and native ranges. (A.3) Performance at the evaluated experimental temperatures in Experiment 1 of European and native ranges. (B) Important variables selected by the regression RF analysis performed with European and native experimental data. Explained variance in each model is exposed at the bottom extremes of the figure.

of *C. edulis* counterbalanced this alteration at the redox state by modifications in the photoprotective system. The alterations in Chl *a*/*b* ratio, total chlorophyll content and carotenoid content in *C. edulis* reflect photo-oxidative stress at the thylakoid membrane. It is known that changes in the pigment composition of light-harvesting complexes allow the reduction of absorbed light (with chlorophyll *a* degradation and lower Chl *a*/*b*) and the increase in energy dissipation through non-photochemical quenching via the xanthophylls, with the deepoxidation of violaxanthin to anteraxanthin and zeaxanthin (the VAZ cycle) preventing excess energy at the chloroplast (Demmig-Adams and Adams, 1996). Degradation of chlorophyll, carotene and xanthophylls has been described as a common response to chilling, revealing cold sensitivity (Kingston-Smith *et al.*, 1999). With the exception of Zx, all other carotenoids content decreased in response to the cold spell. Zeaxanthin is known to play a role in thermal dissipation though the VAZ cycle, but an antioxidant role has also been described for this molecule (Havaux *et al.*, 2007), which might explain the slight increase in Zx content. The xanthophyll cycle is known to act in some overwintering evergreens during winter as a mechanism to avoid winter photoinhibition (Adams *et al.*, 2004). *C. edulis* significantly increased the DPS in response to the cold spell revealing that both ranges use thermal dissipation as quenching mechanisms withstanding low temperatures, supporting our third hypothesis that *C. edulis* has physiological mechanisms to withstand chilling. Previous studies have reported *C. edulis* photoprotective response during winter in the invasive range under natural conditions, concurring with the described increased tolerance to chilling (Fenollosa *et al.*, 2017).

The chlorophyll and VAZ decrease and the increase in the chl *a*/*b* ratio and the DPS were found sooner (at lower chilling intensities) in native *C. edulis* individuals, showing significant differences between ranges at the arrival of the cold spell. Moreover, the transient increase in α-Toc of the native individuals with the arrival of the cold spell, suggests that this molecule might mitigate an oxidative burst derived from the sudden changes in temperature ranges, as this lipophilic antioxidant has an important role scavenging oxygen singlet, preventing lipid peroxidation (Munné-Bosch, 2005). The fact that no alterations in this compound were found under controlled conditions may be a consequence of gradually dropped temperatures, allowing the plants to acclimate to chilling, as chilling sensitivity is described as being dependent on the duration of the stress (Chen *et al.*, 2014). The differential response of invasive and native individuals was also supported by both classification and regression random forest analysis, as ecological performance was predicted with different variables for introduced and native models. Regarding our second hypothesis, we conclude that invasive and native individuals of *C. edulis* respond differently to chilling.

Our results of the ecological niche dynamics suggest an introduced niche expansion of *C. edulis* into colder regions, supporting out first hypothesis that introduced and native *C. edulis* have different climatic niches, as similarity and equivalence between niches were significantly discarded. Moreover, almost all introduced areas in Europe are totally unexpected when modeling species distribution considering native occurrences, exposing the importance of this different physiological response. Despite the generally assumed importance of climate match between the native and introduced regions for invasive success (Keane and Crawley, 2002; Wan and Bonser, 2016), we found evidence of differentiated responses to temperature between individuals from native and introduced ranges of *C. edulis* that are consistent with the observed niche shift, supporting our fourth hypothesis that species expansion has occurred thanks to a differential physiological response of the introduced and native ranges. High niche stability might be determinant for the species' naturalization, but increased cold tolerance may be determinant for this species' expansion and its aggressive behaviour in its introduced range. In spite of the fact that niche conservatism between native and invasive ranges has been largely assumed and niche shifts in plant species may be rare (Petitpierre *et al.*, 2012), recently functional shifts have also been described in invasive species such as *A. pseudoplatanus*, which presented a differential plasticity in response to shade tolerance (Shouman *et al.*, 2017). Invasive *Acacia* and *Eucalyptus* trees have also broader physiological niches than native ones (Higgins and Richardson, 2014). Climatic niche shifts in invasive species have also been described for *Centaurea maculosa* (Broennimann *et al.*, 2007) and for different exotic species in Australia (Gallagher *et al.*, 2010). The different physiological response observed in *C. edulis* toward greater chilling tolerance contributes to discarding the niche conservatism hypothesis, and suggests genetic differentiation between ranges. This is also supported by a recent study contrasting native and invasive individuals of *C. edulis*, which reported that this species' capacity of division of labor may have been subjected to evolutionary adaptation in the invaded range (Roiloa *et al.*, 2016). Rapid evolutionary changes such as genetic drift and inbreeding in founder populations may contribute to explaining invasive niche shifts contributing to local adaptation (Sork, 2017). Many invasive plants appear to grow more vigorously in their introduced than in their native range, and genetic and phenotypic differentiation has been described for some species between ranges (Bossdorf *et al.*, 2005). Over the last decade, several studies have demonstrated that rapid adaptation can occur in short time-scales and fuel up the expansion of invasive species into new regions (Sax *et al.*, 2007). A recent study with the clonal invasive plant *Alternanthera philoxeroides* in China contrasted individuals from the central portion and the northern edge of the range of this species (where it has expanded recently) and found genetically based differential cold tolerance between individuals (Liu *et al.*, 2019). As purposed by the authors, both genetic and epigenetic changes may have a role on this fast genetic differentiation. Epigenetic contribution when facing novel environmental conditions encountered with range expansion may be especially important in clonal species, as they have reduced epigenetic resetting due to the lack of meiosis (Verhoeven and Preite, 2014). Indeed, epigenetic changes together with senescence of plant parts and phenotypic variation have been purposed as parental generation heritance pathways contributing to *C. edulis* success (Fenollosa *et al.*, 2016).

Although the climatic niche expansion towards colder zones goes together with the found differential chilling tolerance, the fact that *C. edulis* is able to grow in zones out of its native distribution range may be assisted by a high propagule pressure. Indeed, the lower explained variance of the performance model for the introduced *C. edulis* population (27% in front 62% in the native model) (Fig. 6) suggests that other factors besides cold tolerance are determining the species presence at the introduced range. The study of the invasive marine clonal macrophyte *Caulerpa* *cylindracea* revealed that propagule pressure together with competence and vegetative growth are determining species invasive success (Balestri *et al*., 2018). The introduction and initial spread of *C. edulis* follows the horticultural industry both also was used for soil and sand dune stabilization (Campoy *et al*., 2018). As evaluated by Bazzichetto *et al*. (2018), this species is now widely planted as an ornamental plant in summerhouses with gardens directly facing coastal dunes providing local input of propagules that constantly supports invasion. Moreover, artificial surfaces had been identified as important propagule sources for *C. edulis* (Carranza *et al*., 2010). The constant human introduction and maintenance of a species out of its native range may promote an increased selection pressure (Willi and Van, 2019) that could have originated the increased chilling tolerance in *C. edulis*. Bottleneck effect and the intrinsic capacity of the species to adapt rapidly to novel environmental conditions though death and growth cycles, clonal growth and epigenetics (Fenollosa *et al*., 2016), could have had also a role on the chilling tolerance acquisition of this species. Moreover, a high hybridization capacity has been described for species of the genus *Carpobrotus* (Albert *et al*., 1997; Suehs *et al*., 2004a, 2004b). In California, hybridization between *C. edulis* and *C. chilensis* contributes to invasion success enhancing species plasticity (Weber and D'Antonio, 2000). In Europe, both *C. edulis* and *Carpobrotus acinacifformis* are considered invasive in some countries and some studies have pointed out the existence of hybridized individuals across the territory, referring to them as *C. affine acinacifformis* (Suehs *et al*., 2004b). There are no studies evaluating the genetic differentiation and genotype prevalence across all the introduced territory of *Carpobrotus* in Europe, but based on species occurrence descriptions, *C. acinaciformis* has a limited distribution in comparison to *C. edulis* (see Fig. 3 in Campoy *et al*., 2018). The found differential physiological response between the analysed native and European *C. edulis* populations in this study goes in accordance with the observed niche shift based on the species occurrence, suggesting that this acquired chilling tolerance may be potentially consistent across the introduced range. However, broad genetic studies are needed to characterize the genetic content of *C. edulis* phenotypes across all its introduced range and understand the role of propagule pressure on the survival of the introduced individuals at the limit of the species distribution.

SDMs have contributed to the projection of invasive species' spatial dynamics due to climate change, revealing some niche shifts that may require changes in management policies (Romero-Alvarez *et al*., 2017; Wei *et al*., 2017). As climate change will increase the intensity, frequency and duration of abnormally low and high temperatures (Christensen *et al*., 2007), a broad ecological niche may be a key trait to respond to climate change, and therefore more effort should be made to prevent *C. edulis* expansion. However, management strategies may not only consider species growth and expansion but also the effective reduction of propagule pressure, as it may have a determinant role on species expansion and rapid adaptation in the introduced range.

Conclusions

Our study found evidence of physiological differentiation towards an increased chilling tolerance between individuals from the invaded and native ranges of the species *C. edulis*, which may explain the introduced climatic niche shift to colder climates observed. The species increased chilling tolerance together with a high propagule pressure has allowed its extensive invasion in Europe.

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References

Adams W, Zarter CR, Ebbert V, Demmig-Adams B (2004) Photoprotective strategies of overwintering evergreens. *Bioscience* 54: 41–49.

Albert ME, D'Antonio CM, Schierenbeck KA (1997) Hybridization and introgression in *Carpobrotus* spp. (Aizoaceae) in California. I. Morphological evidence. *Am J Bot* 84: 896–904.

Amaral JS, Casal S, Torres D, Seabra RM, Oliveira BPP (2005) Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method. *Anal Sci* 21: 1545–1548.

Balestri E, Vallerini F, Menicagli V, Barnaba S, Lardicci C (2018) Biotic resistance and vegetative propagule pressure co-regulate the invasion success of a marine clonal machrophyte. *Sci Rep* 8: 16621.

Bazzichetto M, Malavasi M, Barták V, Rosario Acosta AT, Moudrý V, Carranza ML (2018) Modeling plant invasion on Mediterranean coastal landscapes: an integrative approach using remotely sensed data. *Landsc Urban Plan* 171: 98–106.

Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144: 1–11.

Briscoe Runquist RD, Lake T, Tiffin P, Moeller DA (2019) Species distribution models throught the invasion history of palmer amaranth predict regions at risk of future invasion and reveal challenges with modeling rapidly shifting geographic ranges. *Sci Rep* 9: 2426.

Broennimann O, Di Cola V, Guisan A (2016) *Ecospat: Spatial Ecology Miscellaneous Methods. R Package. Version 2.1.0*, http://www.unil.ch/ecospat/home/menuguid/ecospat-resources/tools.html.

Broennimann O *et al.* (2012) Measuring ecological niche overlap from occurrence and spatial environmental data. *Glob Ecol Biogeogr* 21: 481–497.

Broennimann O, Treier UA, Müller-Schärer H, Thuiller W, Peterson AT, Guisan A (2007) Evidence of climatic niche shift during biological invasion. *Ecol Lett* 10(8):701–709.

Campoy JG *et al.* (2018) Monographs of invasive plants in Europe: *Carpobrotus*. *Botany Letters* 165: 440–475.

Cansev A, Gulen H, Celik G, Eris A (2012) Alterations in total phenolic content and antioxidant capacity in response to low temperatures in olive (*Olea europaea* L. "Gemlik"). *Plant Arch* 12: 489–494.

Carranza L, Carboni M, Feola S, Acosta A (2010) Landscape-scale patterns of alien plant species on coastal dunes: the case of iceplant in Central Italy. *Appl Veg Sci* 13: 135–145.

Chen L, Xian H, Miao Y, Zhang L, Guo Z, Zhao X, Li J, Li T (2014) An overview of cold resistance in plants. *J Agron Crop Sci* 200: 237–245.

Christensen JH *et al.* (2007) Regional climate projections. In S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor, HL Miller, eds, *Climate Change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK and New York, NY, USA

Conser C, Connor EF (2009) Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. *Biol Invasions* 11: 349–358.

D'Antonio C, Mahall B (1991) Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. *Am J Bot* 78: 885–894.

Demmig-Adams B, Adams WW (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci* 1: 21–26.

Feld CK, Segurado P, Gutiérrez-Cánovas C (2016) Analysing the impact of multiple stressors in aquatic biomonitoring data: a 'cookbook' with applications in R. *Sci Total Environ* 573: 1320–1339.

Fenollosa E, Roach DA, Munné-Bosch S (2016) Death and plasticity in clones influence invasion success. *Trends Plant Sci* 21: 551–553.

Fenollosa E, Munné-Bosch S, Pintó-Marijuan M (2017) Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*. *Physiol Plant* 160: 185–200.

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int J Climatol* 37: 4302–4315.

Gallagher RV, Beaumont LJ, Hughes L, Leishman MR (2010) Evidence for climatic niche and biome shifts between native and novel ranges in plant species introduced to Australia. *J Ecol* 98: 790–799.

Havaux M, Dall'Osto L, Bassi R (2007) Zeaxanthin has enhanced antioxidant capacity with respect to all other xanthophylls in Arabidopsis leaves and functions independent of binding to PSII antennae. *Plant Physiol* 145: 1506–1520.

Higgins SI, Richardson DM (2014) Invasive plants have broader physiological niches. *PNAS* 111: 10610–10614.

John R, Anjum NA, Sopory SK, Akram NA, Ashraf M (2016) Some key physiological and molecular processes of cold acclimation. *Biol Plant* 60: 603–618.

Joshi S, Tielbörger K (2012) Response to enemies in the invasive plant *Lythrum salicaria* is genetically determined. *Ann Bot* 110: 1403–1410.

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends Ecol Evol* 17: 164–169.

Kearney M, Porter W (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecol Lett* 12: 334–350.

Kingston-Smith AH, Harbinson J, Foyer A (1999) Acclimation of photosynthesis, H_2O_2 content and antioxidants in maize (*Zea mays*) grown at suboptimal temperatures. *Plant Cell Environ* 22: 1071–1083.

Levitt J (1980) *Responses of Plants to Environmental Stresses*. Academic Press, New York

Liao ZY, Scheepens JF, Li WT, Wang RF, Zheng YL (2019) Biomass reallocation and increased plasticity might contribute to successful invasion of *Chromolaena odorata*. *Flora* 256: 79–84.

Liu D, Horvath D, Li P, Liu W (2019) RNA sequencing characterizes transcriptomes differences in cold response between northern and southern *Alternanthera philoxeroides* and highlight adaptations associated with northward expansion. *Front Plant Sci* 10(24): 1–11.

Mittler R (2006) Abiotic stress, the field environment and stress combination. *Trends Plant Sci* 11: 15–19.

Munné-Bosch S (2005) The role of α-tocopherol in plant stress tolerance. *J Plant Physiol* 162: 743–748.

Munné-Bosch S, Alegre L (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. *Planta* 210: 925–931.

Novoa A, González L (2014) Impacts of *Carpobrotus edulis* (L.) N.E.Br. on the germination, establishment and survival of native plants: a clue for assessing its competitive strength. *PLoS One* 9: e107557. doi: 10.1371/journal.pone.0107557.

Peterson AT (2003) Predicting the geography of species' invasions via ecological niche modeling. *Q Rev Biol* 78: 419–433.

Petitpierre B, Kueffer C, Broennimann O, Randin C, Daehler C, Guisan A (2012) Climatic niche shifts are rare among terrestrial plant invaders. *Science* 335: 1344–1348.

Phillips SJ, Anderson RP, Shapire RE (2006) Maximum entropy modeling for species geographic distributions. *Ecol Model* 190: 231–259.

Phillips SJ, Dudík M, Schapire RE (2018) *Maxent Software for Modeling Species Niches and Distributions (Version 3.4.1)*, http://biodiversityinformatics.amnh.org/open_source/maxent/ (Accessed on 15 December 2018)

Pintó-Marijuan M, Munné-Bosch S (2014) Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: advantages and limitations. *J Exp Bot* 65: 3845–3857.

Preston JC, Sandve SR (2013) Adaptation to seasonality and the winter freeze. *Front Plant Sci* 4(167): 1–18.

Roiloa SR, Retuerto R, Campoy JG, Novoa A, Barreiro R (2016) Division of labor brings greater benefits to clones of *Carpobrotus edulis* in the non-native range: evidence for rapid adaptive evolution. *Front Plant Sci* 7(349): 1–13.

Roiloa SR, Rodríguez-Echeverría S, De la Peña E, Freitas H (2010) Physiological integration increases the survival and growth of the clonal invader *Carpobrotus edulis*. *Biol Invasions* 12: 1815–1823.

Roiloa SR, Rodríguez-Echeverría S, Freitas H (2014a) Effect of physiological integration in self/non-self genotype recognition on the clonal invader *Carpobrotus edulis*. *J Plant Ecol* 7: 413–418.

Roiloa SR, Rodríguez-Echeverría S, Freitas H, Retuerto R (2013) Developmentally-programmed division of labour in the clonal invader *Carpobrotus edulis*. *Biol Invasions* 15: 1895–1905.

Roiloa SR, Rodriguez-Echeverría S, López-Otero A, Retuerto R, Freitas H (2014b) Adaptive plasticity to heterogeneous environments increases capacity for division of labor in the clonal invader *Carpobrotus edulis* (Aizoaceae). *Am J Bot* 101: 1301–1308.

Romero-Alvarez D, Escobar LE, Varela S, Larkin DJ, Phelps NBD (2017) Forecasting distributions of an aquatic invasive species (*Nitellopsis obtusa*) under future climate scenarios. *PLoS One* 12: e0180930. doi: 10.1371/journal.pone.0180930.

Sax DF, Stanchowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK (2007) Ecological and evolutionary insights from species invasions. *Trends Ecol Evol* 22: 466–471.

Schoener TW (1970) Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology* 51: 408–418.

Shouman S, Mason N, Kichey T, Closset-Kopp D, Heberling JM, Kobeissi A, Decocq G (2017) Functional shift of sycamore maple (*Acer pseudoplatanus*) towards greater plasticity and shade tolerance in its invasive range. *Perspect Plant Ecol* 29: 30–40.

Simberloff D *et al.* (2013) Impacts of biological invasions: what's what and the way forward. *Trends Ecol Evol* 28: 58–66.

Sork V (2017) Genomic studies of local adaptation in natural plant populations. *J Hered* 109: 3–15.

Suehs CM, Affre L, Médail F (2004a) Invasion dynamics of two alien *Carpobrotus* (Aizoaceae) taxa on a Mediterranean island: I. genetic diversity and introgression. *Heredity* 92: 31–40.

Suehs CM, Affre L, Médail F (2004b) Invasion dynamics of two alien *Carpobrotus* (Aizoaceae) taxa on a Mediterranean island: II. Reproductive strategies. *Heredity* 92: 550–556.

Suzuki N, Mittler R (2006) Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. *Physiol Plant* 126: 45–51.

Thuiller W, Richardson DM, Pysek P, Midgley GF, Hughes GO, Rouget M (2005) Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Glob Chang Biol* 11: 2234–2250.

Verhoeven KJ, Preite V (2014) Epigenetic variation in asexually reproducing organisms. *Evolution* 68(3): 644–655.

Vilà M, Valladares F, Traveset A, Santamaría L, Castro P (eds) (2008) *Invasiones Biológicas*. CSIC, Madrid, Spain

Wan J, Bonser S (2016) Enemy release at range edges: do invasive species escape their herbivores as they expand into new areas? *J Plant Ecol* 9: 636–647.

Warren D, Glor RE, Turelli M (2008) Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution* 62(11): 2868–2883.

Weber E, D'Antonio CM (2000) Phenotypic plasticity in hybridizing *Carpobrotus* spp. (Aizoaceae) from coastal California and its role in plant invasion. *Can J Bot* 77: 1411–1418.

Wei J, Zhang H, Zhao W, Zhao Q (2017) Niche shifts and the potential distribution of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) under climate change. *PLoS One* 12: e0180913.

Willi Y, Van Burskirk J (2019) A practical guide to the study of distribution limits. *Am Nat* 193(6): 773–785.

Discussió general

Discussió general

Les invasions biològiques provoquen un impacte global sobre la biodiversitat que ens protegeix i ens proveeix serveis essencials. El seu impacte és especialment important sobre aquells espais que acullen un nombre elevat d'espècies endèmiques, com ara la conca Mediterrània. Malgrat la gran diversitat d'hipòtesis sobre els factors que condueixen una espècie a esdevenir invasora, el procés d'invasió es basa en la supervivència i adaptació a una àrea fora del rang de distribució natural de l'espècie i, per tant, involucra estratègies de tolerància i resistència a les noves condicions ambientals. Les respostes a l'estrès, la reproducció i la mort defineixen l'estratègia vital d'una espècie i confereixen aquesta perspectiva ecofisiològica que ens proporciona eines per entendre les invasions.

1. L'estrès i la invasió biològica

L'estrès ens posa a prova. El fet de poder o no suportar certes condicions i de quina manera ho fem condiciona la supervivència. L'estrès delimita la distribució biogeogràfica dels éssers vius. L'estudi de les respostes i tolerància a l'estrès esdevé per tant una eina imprescindible per a entendre els mecanismes que acompanyen les espècies invasores, que salten fora els seus límits de distribució naturals. Tant en condicions de baixa disponibilitat de nutrients, poca llum o poca disponibilitat hídrica, l'estrès ha demostrat limitar les invasions (Alpert et al., 2000). Ara bé, estudis més recents apunten que algunes espècies invasores podrien disposar de mecanismes per envair ambients amb limitació de recursos. Una elevada eficiència en l'ús de l'aigua permetria a les espècies invasores una major conservació dels recursos, així com una major inversió en arrels o una fenologia avançada permetrien una major adquisició de recursos, explicant l'èxit invasor en zones amb baixa disponibilitat d'aquests (Funk, 2013). També s'ha descrit que les invasions especialment eficients són aquelles en què l'espècie és capaç d'envair ambients subòptims, però modificar-los perquè esdevinguin favorables

(Cuddington i Hastings, 2004). És important no condicionar processos essencials per sobreviure en resposta a unes condicions determinades, però també ho és l'amplitud de condicions ambientals en què una espècie pot sobreviure. Ser capaç de sobreviure a moltes condicions diferents permet a l'espècie adaptar-s'hi. De fet, l'estudi de Higgins i Richardson (2014) demostra que les plantes invasores tenen una amplitud fisiològica del nínxol major que les natives. Funk (2008) també demostra que la plasticitat en atributs fisiològics i morfològics d'espècies invasores pot proveir un major accés a recursos limitats i afavorir el seu èxit.

1.1. Fent front al Mediterrani: respostes a l'estrès hídric i a les baixes temperatures

Les plantes han evolucionat per superar la pressió de selecció del seu hàbitat, i per això s'assumeix que una història biogeogràfica més llarga contribueix a una major adaptació local (Kawecki i Ebert, 2004). A l'arribar en un indret fora el rang de distribució natural, l'espècie al·lòctona es troba amb unes condicions i dinàmiques ambientals pròpies del lloc d'arribada. La capacitat fisiològica de fer front a aquestes dinàmiques determinarà la seva persistència i la possibilitat d'esdevenir invasora. El clima mediterrani suposa un repte per la duresa de la sequera estival juntament amb la intensa radiació i les elevades temperatures, però també per l'acumulació de dies clars i freds durant l'hivern. Les espècies mediterrànies disposen d'una gran varietat d'estratègies per fer front a aquests estressos (Zunzunegui et al., 2011; Matesanz i Valladares, 2014; Fernández-Marín et al., 2017). Són especialment eficients en fer front a l'estrès hídric gràcies a adaptacions morfològiques que permeten minimitzar la pèrdua hídrica o evitar l'excés de llum així com a gràcies a un eficient sistema de fotoprotecció per minimitzar el dany cel·lular en condicions de falta d'aigua (Gulías et al., 2002; Hernández et al., 2004; Warren et al., 2007; Matesanz i Valladares, 2014; Fernández-Marín et al., 2017). En el cas de la invasora al Mediterrani, *C. edulis*, en el **capítol 1** es va avaluar de quina manera feia front a aquestes condicions ambientals del rang invasor, en comparació a la nativa

Crithmum maritimum, amb qui comparteix hàbitat al Cap de Creus. Els resultats obtinguts apunten al fet que l'espècie sobreviu exitosament a les condicions ambientals locals desplegant respostes fotoprotectores als dos moments més crítics de l'anualitat mediterrània: l'estiu i l'hivern. L'espècie recupera els nivells de tots els marcadors d'estrès analitzats al finalitzar l'anualitat, revelant que es troba adaptada a la dinàmica estacional del Mediterrani. La plasticitat de *C. edulis* a fer ús de diferents mecanismes fotoprotectors li permet fer front a aquests moments crítics de forma igual d'exitosa que l'espècie nativa.

Durant l'estiu mediterrani, en els **capítols 1 i 2** veiem la importància de la variació en el contingut de clorofil·les, en la relació entre la clorofil·la *a* i *b* (Chl *a/b*), en el contingut de xantofil·les, en el DPS i en l'α-tocoferol en *C. edulis* per fer front a l'estrès hídric. Tal com observem en el **capítol 2**, després de 48 dies amb baixa disponibilitat hídrica, tant els ramets senescents (els reproductors, per la senescència monocàrpica deguda a la presència de fruit) com els no senescents, són capaços de recuperar els continguts de clorofil·les gràcies a variacions en el contingut de citocinines, que participen en el procés de recuperació a la sequera revertint la senescència foliar. Aquesta recuperació de la verdor o *re-greening* va acompanyada de la recuperació hídrica foliar l'acabar l'estiu, coincidint amb els nivells d'hidratació alts que presenta l'espècie durant la resta de l'any (**capítol 1**). La regulació de la mida de l'antena és una resposta comuna a molts estressos ja que permet la reducció de l'absorció de llum i minimitza l'acumulació d'energia al cloroplast (Morosinotto i Bassi, 2012). En última instància el contingut de clorofil·les determina la proporció de llum absorbida i per tant el potencial fotosintètic (Esteban et al., 2015; Demmig-Adams et al., 2014). Malgrat la reducció en l'absorció de llum associada a la degradació de clorofil·les en condicions d'estrès hídric, el tancament estomàtic provoca la falta de substrat per la fotosíntesi contribuint a l'acumulació d'energia d'excitació de les clorofil·les. L'elevada inversió en dissipació tèrmica a través del cicle de les xantofil·les i en la síntesi d'antioxidants en *C. edulis* durant el període de sequera permeten reduir les ROS generades com a conseqüència de l'acumulació d'energia al cloroplast. Altres espècies típiques de la conca Mediterrània com

Rosmarinus officinalis o *Cistus salvifolius* també mostren estratègies similars (Munné-Bosch i Alegre, 2000; Grant et al., 2015).

L'estiu mediterrani no és l'únic moment a l'any en què *C. edulis* desplega les seves respostes fotoprotectores. L'hivern, també posa a prova la supervivència de les espècies al Mediterrani. En el **capítol 1**, durant el mes de gener en *C. edulis* creixent en condicions naturals al Cap de Creus, les fredes temperatures, la baixa humitat i l'elevat nombre de dies clars que caracteritzen els hiverns al Mediterrani (Oliveira i Peñuelas, 2004; Ciccarelli et al., 2016) també provoquen canvis fisiològics en aquesta espècie. Algunes espècies mediterrànies pateixen fotoinhibició hivernal en què el cicle de les xantofil·les juga un paper clau (Adams et al., 2004; Míguez et al., 2015). En condicions naturals del Cap de Creus durant l'estudi anual del **capítol 1**, les temperatures van oscil·lar entre els 8ºC i 25ºC de temperatura mitjana, amb gener i febrer com els mesos més freds. Durant l'hivern, en condicions naturals, la hidratació es manté a nivells semblants als observats en condicions controlades a temperatures similars, relativament alta en comparació a la deshidratació patida durant els mesos d'estiu. Observem una baixada en la F_v/F_m, malgrat que aquesta es manté per sobre 0,75, acompanyada de canvis en la relació Chl *a/b*, el contingut de VAZ i luteïna i el DPS. A l'aïllar el factor de la temperatura en els **capítols 4 i 5** en condicions controlades podem aprofundir en les respostes a l'estrès per aquest factor, explorant les respostes per sota la forquilla de temperatures observada en condicions naturals. Els resultats dels estudis en condicions controlades i semicontrolades revelen que *C. edulis* presenta respostes hormonals diferenciades per donar resposta a les baixes temperatures, en funció de la duració i severitat d'aquestes (**capítol 4 i 5**). Malgrat mostrar nivells similars de dessecació foliar, en percebre una baixada de les temperatures de forma sobtada per sota dels 8 ºC, *C. edulis* respon amb un augment en els continguts de SA i una caiguda en el contingut de melatonina, mentre que en permetre un període d'aclimatació amb una baixada progressiva de les temperatures observem un augment transient de melatonina conjuntament amb augments en ABA i citocinines. Sota ambdues condicions observem com l'estrès per baixes temperatures va acompanyat d'un augment en el contingut de carotenoides, així com en el DPS.

Malgrat el contrast entre les condicions ambientals que encontra *C. edulis* a l'estiu i a l'hivern, l'estudi de les respostes fisiològiques de *C. edulis* ens porta a identificar punts comuns com la caiguda en el contingut hídric foliar, els canvis en el contingut de clorofil·les i el desplegament de respostes fotoprotectores (**Figura 3**). La deshidratació és una conseqüència àmpliament descrita en resposta als estressos hídric, salí, osmòtic i per altes i baixes temperatures (Zhang i Sonnewald, 2017). La degradació de clorofil·les i la dissipació tèrmica a través del cicle de les xantofil·les amb l'augment de la pressió d'estrès, així com la seva recuperació en cessar, també són respostes comunes de *C. edulis* tant a l'estrès per baixes temperatures com a l'estrès durant l'estiu. Diversos estudis han explorat els mecanismes de tolerància creuada en condicions de congelació (Temperatura < 0ºC) i estrès hídric, que són possibles gràcies a un gran nombre de mecanismes compartits en resposta ambdós estressos (Beck et al., 2007; Verhoeven et al., 2018). Aquesta dualitat i conservació de mecanismes per a fer front a l'estrès foto-oxidatiu derivat del fred i l'estrès hídric s'ha descrit recentment en *Ramonda myconi*, una espècie paleotropical reviviscent (és capaç de recuperar la seva fisiologia després d'una dessecació completa) que és alhora tolerant a la congelació (Fernández-Marín et al., 2020). L'estudi amb aquesta espècie mostra com a respostes comunes al fred hivernal i a la sequera estival als Pirineus una major fotoprotecció, major dissipació tèrmica a través del cicle de les xantofil·les i una major relació de carotenoids per clorofil·la. Finalment, les variacions en el contingut de citocinines en C. edulis en resposta a la baixa disponibilitat hídrica i les baixes temperatures, especialment la *trans*-zeatina, també juguen un paper dual tant en les respostes a ambdós estressos, malgrat que difereixen entre fulles de ramets reproductors i ramets no reproductors. El seu rol en frenar els processos de senescència actua tant en la recuperació a un estrès hídric sever (**capítol 2**), com en la disminució progressiva de la temperatura a partir dels 8 ºC (**capítol 4**). Aquest resultat podria suggerir un rol transversal de les citocinines en resposta a estressos progressius.

En conjunt, la plasticitat de *C. edulis* per respondre de forma exitosa tant a la baixa disponibilitat hídrica com a les baixes temperatures, ajustant la seva resposta fisiològica en funció de la intensitat i durada d'aquests estressos, li permet colonitzar de forma efectiva les regions mediterrànies. La gran amplitud fisiològica de *C. edulis* es pot relacionar amb l'eficiència dels mecanismes de resposta a l'estrès que presenta, comuns sota condicions ambientals tan diverses com la sequera i les baixes temperatures.

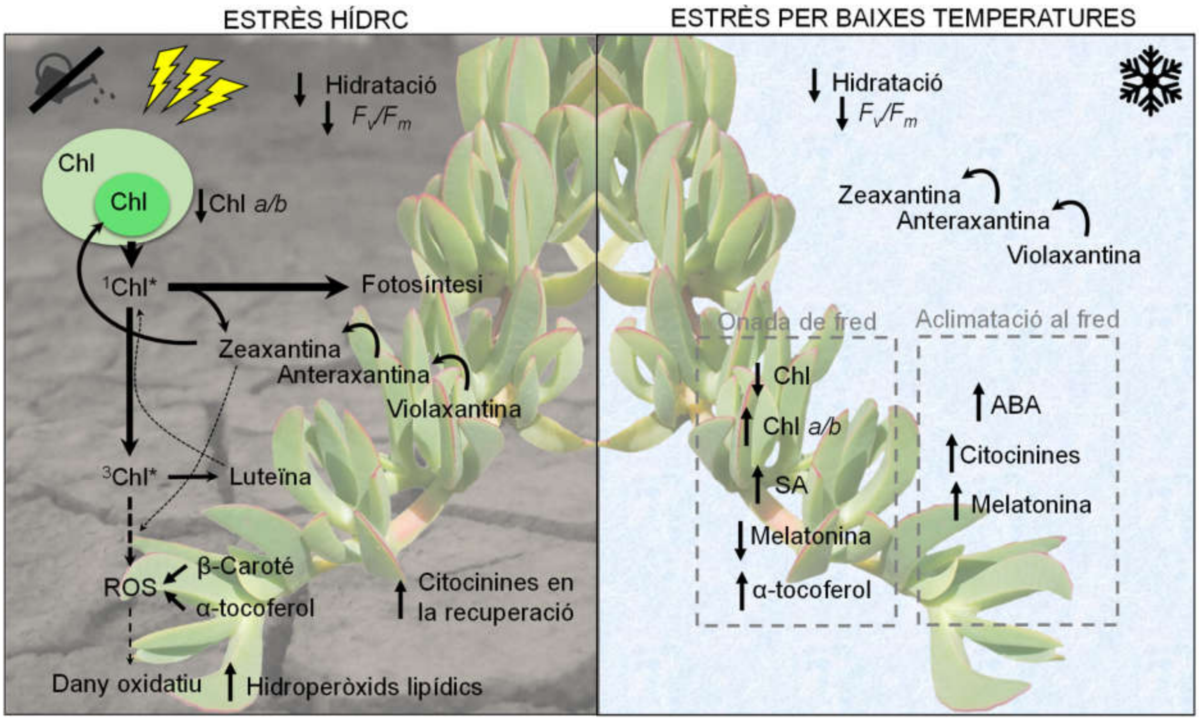

Figura 3. Respostes fisiològiques de *C. edulis* a l'estrès hídric i a les baixes temperatures, els dos estressos clau en la resposta fisiològica al Mediterrani.

1.2. Marcadors d'estrès per entendre la invasió

L'ús de marcadors d'estrès oxidatiu permet entendre els mecanismes de resposta de les plantes al seu ambient i, per extensió, els mecanismes de resposta de les plantes invasores als nous ambients, als quals arriben generant grans impactes. La presència d'una espècie en un hàbitat depèn d'un conjunt de factors, com ara els factors abiòtics, els quals juguen un paper molt important (Grinnell, 1917). El fet que l'hàbitat es trobi dins del nínxol fonamental de l'espècie és un requisit indispensable per la seva presència. Definim el nínxol fonamental d'una espècie com l'hipervolum que ocupa, en termes de factors ambientals que en permeten el benestar (Hutchinson, 1957). Tant l'acumulació d'antioxidants com l'ajust osmòtic constitueixen eines fisiològiques que poden ajudar a entendre l'èxit invasor, així com altres atributs ecofisiològics en resposta a estressos ambientals (Pintó-Marijuan i Munné-Bosch, 2013). La recerca desenvolupada en *C. edulis* utilitzant marcadors d'estrès ens ha permès posar en pràctica la utilitat d'aquests marcadors per a l'estudi de les invasions biològiques amb diferents propòsits: comparar amb natives coexistents per avaluar l'adaptació, comparar entre rang natiu i invasor a nivell funcional per entendre els mecanismes de la invasió i entendre la capacitat de resposta de l'espècie a condicions ambientals (**Figura 4**). Els diferents marcadors d'estrès utilitzats han permès entendre l'estratègia de *C. edulis* per fer front a les dinàmiques ambientals del rang invasor en els **capítols 1 i 2,** però també han revelat la diferenciació funcional envers una major tolerància a les baixes temperatures que presenten els individus del rang invasor i que ha permès a aquesta espècie expandir-se per Europa tal com es presenta en el **capítol 5**. Caracteritzar la tolerància a estressos ambientals pot permetre modelitzar les zones d'hàbitat potencial actuals des d'una perspectiva mecanicista, però també sota escenaris de canvi climàtic. En concret, els marcadors d'estrès fotooxidatiu, poden contribuir a una millor comprensió dels límits del nínxol fisiològic de les espècies invasores a través d'entendre la seva tolerància a l'estrès tal com s'exposa a **l'annex 1**.

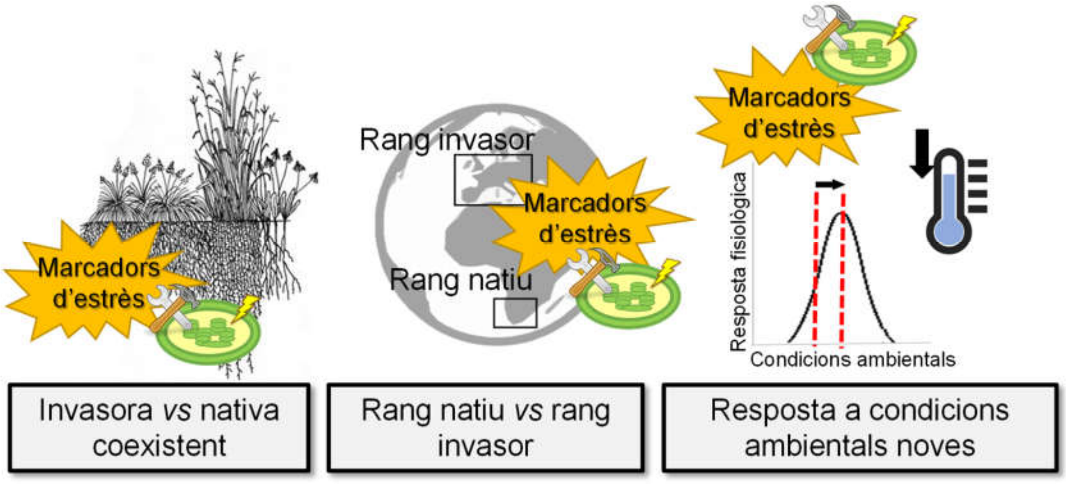

Figura 4. Marcadors d'estrès fotooxidatiu en l'estudi de les invasions per a comparar les respostes entre espècies invasores i natives, contrastar individus del rang natiu i invasor i avaluar la capacitat de resposta a noves condicions ambientals.

2. La reproducció sexual en la invasió d'una espècie clonal

2.1. L'estratègia reproductora en la invasió

L'estratègia reproductora juga un paper clau en la persistència d'espècies clonals en un hàbitat, ja que facilita l'adaptació a través de la variació genètica i en la seva expansió a través de la dispersió a llarga escala (Schmid, 1990; Eriksson, 1992). *C. edulis* presenta una estratègia monocàrpica a nivell de ramet. La floració no ocorre en tots els ramets d'un genet, i només aquests entren en senescència remobilitzant els nutrients cap al fruit fins a madurar i dessecar-se. La reproducció és única en la vida del ramet, però és múltiple a nivell de genet. Així doncs *C. edulis* és una espècie perenne monocàrpica a nivell de ramet i policàrpica o iteròpara a nivell de genet. Una estratègia similar s'ha proposat en algunes espècies del gènere *Agave* que tradicionalment s'havien classificat com a monocàrpiques i constituïen el típic exemple d'aquesta estratègia (Simcha, 2017). La comparació de dues espècies invasores taxonòmicament semblants (*Tithonia diversifolia* i *T. rotundifolia*) però divergents en la seva estratègia reproductora (policàrpica i monocàrpica respectivament) mostra com la monocàrpica arriba més ràpid a la maduresa reproductora, produeix llavors grans i mostra un gran esforç reproductor, mentre que la policàrpica produeix llavors petites i presenta també reproducció asexual (Muoghalu, 2008). Un cicle de vida ràpid s'ha relacionat amb la capacitat invasora (Baker, 1965), però la monocàrpia implica una gran inversió en la reproducció, ja que almenys en els casos de monocàrpia "pura", la contribució d'un individu es limita a un sol acte reproductiu (Gadgil i Bossert, 1970), una aposta del tipus *bed-hedging* (tot a un sol niu). *C. edulis* escapa dels riscos del *bed-hedging* associat a la monocàrpia a través d'aquesta divisió de tasques entre ramets vegetatius i ramets reproductors. Aquesta estratègia permetria allargar la vida del genet tal com s'ha descrit en l'espècie d'alta muntanya *Saxifraga longifolia* que segueix una estratègia monocàrpica lligada a la roseta (Cotado i Munné-Bosch, 2020). Ara bé, aquests patrons de reproducció a nivell subindividual han estat pobrament descrits i qüestionen la definició de clonalitat (Klimešová et al., 2020).

Una elevada inversió en reproducció s'ha relacionat amb la capacitat d'esdevenir una espècie invasora (Jelbert et al., 2015). Una major inversió en la reproducció resulta una estratègia altament adaptativa en ambients competitius (Bonser, 2013). La comparació de vint parells d'espècies de la regió mediterrània francesa va demostrar que aquelles que tenen un rang de distribució més ampli presenten una major producció de llavors, en contrast amb espècies taxonòmicament properes amb nínxols restringits (Lavergne et al., 2004). En el cas d'espècies invasores, la comparació de *Pinus contorta* entre el seu rang natiu i introduït ha revelat un augment en l'esforç reproductor als rangs introduïts (Taylor et al., 2016). En aquest sentit, l'estudi de Wu et al., (2005) demostra que la fecunditat per planta pot permetre predir el fet que una espècie esdevingui invasora. Els trets reproductors permeten també predir quines espècies exòtiques arriben a ser invasores a la República Txeca, demostrant que dins el model generat amb un arbre de classificació, gran part de les invasores tenen llavors més petites i també elevades produccions de llavors (>38.000 llavors/m^2) (Moravcová et al., 2015). *C. edulis* té també llavors petites amb elevada viabilitat i una elevada producció de llavors. Considerant les dades recollides en *C. edulis* sobre l'esforç reproductor en el **capítol 2** (entre 13 i 153 fruits/m^2) i les dades del nombre de llavors per fruit en el **capítol 3** (entre 60 i 2.500 llavors/fruit) podem estimar que la producció de llavors podria estar compresa dins l'aclaparador interval d'entre 750 i 380.000 llavors/m^2, amb unes 83.000 llavors/m^2 de mitjana, superant la producció de llavors de tall en la classificació d'invasores de Moravcová et al. (2015) de 38.000 llavors/m^2. A més, la gran inversió en la reproducció sexual la veiem també en l'elevada longevitat de les llavors de *C. edulis* que formen un banc de llavors permanent. La formació de bancs de llavors al sòl s'ha relacionat amb la capacitat invasora, ja que dota d'una gran capacitat de persistència a llarg termini, així com una elevada resiliència davant de les pertorbacions (Gioria et al., 2012).

El creixement clonal també juga un paper clau en l'estratègia reproductora de *C. edulis* (Roiloa, 2019). La clonalitat s'ha definit com un dels atributs que podria definir la planta invasora ideal, ja que permet a l'espècie reproduir-se i evitar els riscos de la reproducció sexual com la depressió endogàmica, malgrat comptar inicialment amb pocs individus (Pysek, 1997; Song et al., 2013). Els individus del rang invasor de l'espècie clonal *Spartina artenifolia* mostren més creixement i reproducció asexual que els individus del rang natiu (Shang et al., 2015). S'han obtingut també resultats semblants en altres espècies invasores clonals com *Achillea millefolium* i *Hypericum perforatum*, malgrat que *Hieracium pillosella* no mostra diferenciació entre el creixement clonal entre els rangs natiu i invasor (Beckmann et al., 2009). Al rang natiu, *Alternanthera philoxeroides*, produeix fruits i llavors viables, mentre que no trobem llavors viables al rang invasor d'aquesta espècie, on es reprodueix vegetativament a partir de fragments (Pan et al., 2007). Com a espècie clonal, *C. edulis* es beneficia també de l'habilitat de la divisió de tasques gràcies a la integració fisiològica entre ramets, que els permet compartir recursos, integrant així l'heterogeneïtat de recursos en l'espai (Roiloa et al., 2013; 2019). La divisió de tasques beneficia molt més els individus del rang invasor que els del rang natiu, suggerint que aquest atribut ha estat sotmès a adaptació evolutiva durant el procés d'invasió (Roiloa et al., 2016). A més a més, en comptes de limitar la dispersió de l'espècie, la capacitat de creixement clonal de *C. edulis* la dota d'una estratègia de dispersió asexual a llarga escala (fins a 250 km) gràcies a l'elevada capacitat de resistència flotant al mar i la capacitat d'arrelament posterior dels fragments de *C. edulis* (Souza-Alonso et al., 2020).

2.2. Reproducció sexual i asexual: compromís o sinergia?

La teoria de la història de vida i el principi de repartició de recursos assumeixen que la inversió en una funció vital (créixer, reproduir-se, sobreviure) és en detriment de la inversió en altres funcions (Levins, 1968; Stearns, 1992). La correlació inversa entre el creixement vegetatiu i la producció de fruits en plantes i entre el creixement i la reproducció en animals suggereix un compromís entre la reproducció sexual i el creixement (Harper, 1977; Calow, 1979). El creixement clonal ofereix avantatges i inconvenients en la dispersió, l'establiment, la persistència i l'expansió (Pysek, 1997). En una espècie clonal, la producció de ramets incrementa la capacitat del genet per fer més llavors en el futur, així com reduir el risc de mortalitat. Ara bé, la falta de reproducció sexual implica una capacitat de dispersió limitada i la falta de mecanismes de generació de variabilitat genètica, fent a les espècies més susceptibles d'acumular mutacions deletèries (Lynch et al., 1993). La predominança poblacional de la reproducció clonal podria permetre que mutacions que reduïssin la fertilitat conduïssin a la disfunció sexual (Barret, 2015). La producció de llavors permet a un genet l'oportunitat de dispersar-se per colonitzar noves àrees, potser més riques i segures, i pot ser l'estratègia més efectiva si les taxes de mortalitat del genet o ramet són elevades (Hartnett, 1987). D'aquesta manera, les plantes clonals afronten compromisos entre invertir en creixement i assegurar la supervivència, o invertir en reproducció, malgrat que pugui suposar la mort (Gardner i Mangel, 1999). La decantació de la balança dependrà de la probabilitat de mortalitat. La reproducció asexual s'ha descrit com a estratègia més comuna en ambients estressants (Pluess i Stöcklin, 2005). *Hieracium pilosella* inverteix en reproducció vegetativa per maximitzar la persistència, mentre que, si hi ha nínxols buits, inverteix en colonitzar l'espai amb una elevada producció de llavors (Stöcklin i Winkler, 2004). D'aquesta manera, la plasticitat en l'estratègia reproductora constitueix un bressol per fer front a l'estocasticitat ambiental (Bårdsen et al., 2011). La gran variabilitat observada en *C. edulis* en els atributs relacionats amb la reproducció sexual en el **capítol 3** defineix dinàmiques en el banc de llavors diferenciades entre diferents poblacions. Aquesta plasticitat podria permetre a l'espècie una major capacitat d'adaptació local a ambients diferenciats. S'ha descrit que *C. edulis* és lleugerament agamospèrmica és a dir, pot produir llavors per via asexual (Vilà et al., 1998; Suehs et al., 2004). Malgrat reduir la variabilitat genètica al saltar el procés de recombinació, la plasticitat en aquest mecanisme permetria la fixació de combinacions genètiques a través de múltiples generacions, promovent una ràpida adaptació local tal com s'ha descrit amb espècies del gènere *Cenchrus*, a les que l'agamospèrmia permetria la reproducció fins i tot sota condicions estressants (Kumar et al., 2019).

El compromís entre reproducció sexual i asexual es veu també reflectit en el que anomenem 'cost de la reproducció' que definim com la pèrdua en potencial reproductiu futur degut a l'actual inversió en reproducció (Jönsson, 2000). Els costos de la reproducció normalment apareixen encoberts per mecanismes compensatoris i una aproximació per a mesurar-los és la manipulació experimental per evidenciar els efectes de la repartició reproductiva a altres funcions (Obeso, 2002). Dues de les aproximacions que planteja Obeso (2002) són testades en el **capítol 2**: l'eliminació del fruit i l'avaluació d'un gradient d'individus amb diferent esforç reproductor. A més, també podem visualitzar els costos de la reproducció a nivell de ramet, al diferenciar els que tenen càrrega reproductora (ramets reproductors) i els que no, tal com s'explora als **capítols 1 i 2**. Sota condicions de limitació de recursos, s'ha descrit que la reproducció sexual provoca la disminució de la capacitat fotosintètica de les plantes (Sánchez-Vilas i Retuerto, 2011). Aquest és també el cas de *C. edulis*, ja que, a nivell de ramet, veiem com aquells amb càrrega reproductora presenten menor eficiència fotosintètica. A més, els ramets reproductors, que presenten menor contingut de clorofil·les, major acumulació de compostos fotoprotectors i major estrès oxidatiu són més sensibles a l'estrès que els ramets sense aquesta càrrega reproductora. Aquests costos semblen ser irreversibles, ja que l'alliberació de la càrrega reproductora no promou canvis, tal com veiem en el **capítol 2**. Ara bé, l'esforç reproductor en *C. edulis* no té un efecte únicament sobre els ramets reproductors sinó que en el **capítol 2** també veiem com s'estableixen relacions entre la resposta fisiològica a l'estrès hídric i la càrrega reproductora suggerint una important connexió entre ramets reproductors i ramets vegetatius. En un arbre, la necessitat de mantenir branques no reproductores vindria explicat per la necessitat de manteniment després de la reproducció (Obeso, 2002). La hipòtesi de l'especialització de les branques planteja l'especialització a activitats particulars com la reproducció, el manteniment o l'exploració de l'espai (Hasegawa i Takeda, 2001). En aquest sentit, l'estudi de Van Drunen i Dorken (2012) planteja que la majoria de compromisos que s'han definit entre reproducció sexual i clonal s'han fet a nivell de ramet i no de l'individu sencer, mostrant que en els casos en què es considera el cost de la reproducció a nivell de genet, aquest no s'observa.

3. El paper de la senescència en la supervivència

La filosofia al voltant del sentit de la mort va més enllà de l'ecofisiologia vegetal. L'aparició de la vida ha anat lligada al seu final. I és que el que ha portat a les espècies a ser com són actualment ha estat la vida, però també la mort.

Quan Charles Darwin va presentar "*L'origen de les espècies"* l'any 1859, una de les crítiques que va rebre a la seva teoria de la selecció natural va ser el sentit de la mort o l'envelliment. Se li va rebatre que la mort no tenia sentit, que no era adaptativa i segons les seves propostes els individus haurien de ser immortals, ja que la longevitat limitada no seria seleccionada. En plantes perennes la senescència no s'ha demostrat. L'estudi de Baudisch et al. (2013) sobre 290 angiospermes va descriure que el 93 % no mostrava signes de senescència o, fins i tot, presentava senescència negativa. Malgrat aquest resultat, l'estudi de la immortalitat presenta diferents complicacions experimentals com la dificultat en la determinació de l'edat en els estudis transversals, o bé l'elevada temporalitat dels estudis longitudinals (Roach i Smith, 2019).

Tant Alfred Russel Wallace com August Weismann van presentar els primers arguments per explicar l'origen i el manteniment de la senescència basant-se en què aquesta, al reduir la longevitat, accelera el recanvi generacional, fet que facilita l'adaptació a ambients canviants a nivell poblacional (Wallace, 1865; Weismann, 1889). August Weismann va presentar la mort com a tret genètic beneficiós per a l'espècie, programada biològicament. Tanmateix, aquestes propostes també van rebre crítica: Com és que els individus que moren tenen més possibilitats de passar aquest tret als descendents?

Amb els avenços científics i tecnològics, han anat apareixent diferents teories, la majoria no excloents, sobre la senescència en els éssers vius. Per exemple, Medawar (1952) va proposar que l'envelliment ocorre per acumulació de mutacions deletèries i Hoarman (1992) per l'acumulació de radicals lliures i el dany oxidatiu. Williams (1957) presentava el pleiotropisme antagònic que planteja que aquells gens beneficiosos per a l'adaptació primerenca són deleteris en edats posteriors i per això són seleccionats, a favor del vigor en la joventut. També Hayflick (1965) va demostrar una relació entre la replicació cel·lular i la longevitat, definint un límit en el nombre de replicacions cel·lulars. Malgrat el desenvolupament de noves teories que explicarien l'envelliment, moltes no s'han pogut demostrar a nivell d'individu en plantes perennes, probablement per la dificultat del seu estudi, però també per la dificultat d'escalar des de la senescència cel·lular a la de l'individu degut al seu complex i plàstic programa de desenvolupament.

3.1. Senescència per la supervivència a nivell foliar

A nivell d'òrgan, la senescència pot jugar un paper adaptatiu. Per exemple, s'ha descrit que en resposta a l'estrès hídric, la senescència foliar té un rol adaptatiu en el reciclatge de nutrients cap a fulles més productives (Munné-Bosch i Alegre, 2004). La reducció del contingut de clorofil·les associat a la senescència permet, per una banda, reduir els efectes potencials de la formació de $^1O_2$ als tilacoides i la quantitat de llum absorbida, i per altra banda, contribueix a augmentar les relacions de molècules fotoprotectores i antioxidants per unitat de clorofil·la, fet que també regula la capacitat antioxidant amb relació a la llum absorbida (Kyparissis et al., 1995; Krieger-Liszkay, 2005; Takahashi i Badger, 2011). Al Mediterrani, la senescència induïda per sequera és una resposta d'escapament comuna en algunes plantes típicament mediterrànies com *Lavandula stoechas*, *Rosmarinus officinalis*, *Stipa tenacissima* o *Pistacia lentiscus* (Munné-Bosch i Alegre, 2000; Balaguer et al., 2002; Munné-Bosch i Peñuelas, 2004). En *C. edulis* s'ha observat, en resposta a l'estrès hídric en els **capítols 1 i 2,** una caiguda en el contingut de clorofil·les que apunta a senescència foliar induïda per estrès hídric. Tot i això, aquesta pèrdua de les clorofil·les és reversible a l'acabar l'estiu, tant en els ramets sense càrrega reproductora com en els ramets que sí que tenen càrrega reproductora i es troben, per tant, en un procés de senescència associada a la reproducció. En aquests ramets senescents, l'estrès hídric promou una caiguda en el contingut de clorofil·les que accelera la senescència, però, en recuperar l'estat hídric, s'observa la reversió del procés, un *re-greening*, mediat pels continguts de citocinines a la fulla. En diverses espècies s'ha descrit que l'estrès durant el període de remobilització de nutrients cap al fruit accelera aquest procés (Yang i Zhan, 2006). La plasticitat de *C. edulis* de recuperar-se de l'estrès, recuperant els nivells de clorofil·les anteriors, li permet reprendre l'absorció de llum i l'assimilació de carboni, maximitzant així el creixement dels ramets no senescents i la remobilització de nutrients cap a les llavors en ramets senescents.

3.2. Senescència per la supervivència a nivell de ramet

Tal com s'ha presentat anteriorment, la senescència en *C. edulis* a nivell de ramet va associada a la seva reproducció i és, per tant, una planta monocàrpica a nivell de ramet. La teoria de l'esforç reproductiu de Rose (1991) plantejava que la reproducció sexual podria haver sorgit en l'evolució suposant un cost en la longevitat, ja que en molts organismes s'observa el deteriorament fisiològic després de la reproducció. En la majoria d'espècies animals, la senescència s'accentua amb la fi de l'etapa reproductora, moment en què l'individu perd valor reproductor i en què la pressió de selecció podria deixar de tenir importància. Seguint la teoria del gen egoista, l'objectiu vital de perpetuar els propis gens queda complert amb la reproducció (Dawkins 1976). Això es fa especialment palès amb espècies monocàrpiques, que inverteixen tots els recursos en la reproducció, que provoca la mort de l'individu. En el cas de *C. edulis*, cal destacar que la seva estratègia el porta a deixar més herència que la genètica a les següents generacions. *C. edulis* és un disruptor dels processos geoquímics del sòl, ja que altera el pH, el contingut de nutrients, la humitat i l'activitat microbiana, a través de la gran quantitat de necromassa que l'espècie diposita al morir (Conser i Connor, 2009; Novoa et al., 2014; Vieites-Blanco i González-Prieto, 2018a; Vieites-Blanco i González-Prieto, 2018b; Rodríguez-Caballero et al., 2020). Tal com s'exposa a **l'annex 2**, la mort dels ramets reproductors comporta el dipòsit d'una gran quantitat de matèria orgànica al sòl, que inhibeix la germinació d'espècies natives (Novoa i Gonzalez, 2014), malgrat millorar la retenció d'aigua del sòl. La integració fisiològica i divisió de tasques en *C. edulis* permet integrar l'heterogeneïtat en la disponibilitat d'aigua (Roiloa et al., 2013). Gràcies a aquesta capacitat, *C. edulis* pot estendre ramets sobre sòl inhòspit que, a través de diversos cicles de creixement i senescència, esdevindrà sòl favorable per al creixement de les noves generacions (*Grow and die strategy*, **annex 2**). D'aquesta manera, s'estableix una retroalimentació positiva sòl-planta que permet l'establiment, la persistència i l'expansió de l'espècie invasora (de la Peña et al., 2010), qualitats que la defineixen com a espècie enginyera (Cuddington i Hastings, 2004). En aquest sentit, la retroalimentació en la relació sòl-planta s'ha destacat com un dels mecanismes que podria explicar l'abundància i persistència d'espècies invasores (Reinhart i Callaway, 2004). En el cas de la liana invasora *Euonymus fortunei*, la retroalimentació positiva en la relació sòl-planta s'ha demostrat que promou la dominància d'aquesta espècie per sobre espècies natives amb què coexisteix (Smith i Reynolds, 2015).

Què deixa *C. edulis* a les següents generacions? La senescència de *C. edulis* associada a la reproducció a nivell de ramet és beneficiosa per a l'espècie, ja que ofereix en herència més que la genètica, ofereix també una herència que podríem denominar física o patrimonial. Ofereix unes millors condicions de partida, de manera que les noves generacions tindrien més possibilitats de sobreviure sobre la capa de necromassa. Tal com s'esquematitza a **l'annex 2**, *C. edulis* transmet a les següents generacions nova variabilitat genètica a través de la recombinació durant la reproducció sexual, el propi fenotip i els efectes epigenètics a través del genotip meristemàtic. La contribució epigenètica en espècies clonals és especialment important, facilitant l'adaptació a noves condicions ambientals durant l'expansió en el rang invasor (Verhoeven i Preite, 2013). S'ha demostrat que gradients ambientals provoquen diferències epigenètiques i expressió genètica diferencial en pollancre d'idèntic genotip, transmetent-se a les següents generacions (Raj et al., 2011). A banda d'aquests tres contribuïdors a l'herència genètica de les següents generacions, *C. edulis* a través dels cicles de creixement i mort deixa també béns materials: un sòl més habitable que reté l'aigua i exclou les espècies natives.

3.3. Senescència per la supervivència a nivell de genet

Fins ara s’ha exposat el rol adaptatiu de la senescència a nivell foliar i a nivell de ramet, però no a nivell de l’individu sencer. S’ha descrit que la modularitat en plantes, és a dir, la capacitat de regenerar les parts del cos com fulles i arrels per tal de descartar òrgans envellits a favor de teixits nous, podria ser el mecanisme que permetés a les plantes escapar del deteriorament amb l’edat (Dahlgren i Roach, 2017; Munné-Bosch, 2018). En organismes modulars, el creixement és un balanç entre organogènesi i reclutament, escapant així del deteriorament amb l’edat gràcies a la constant renovació (Thomas et al., 2013). Els cicles de mort i creixement podrien permetre a *C. edulis* reiniciar el sistema i gaudir d’una llarga longevitat a nivell de genet. L’elevada longevitat de grans arbres s’ha associat amb la modularitat que permet el renovament d’òrgans i la construcció sobre estructures mortes que permeten el reciclatge d’un eficient sistema vascular (Munné-Bosch, 2018). Tal com s’ha presentat als apartats anteriors i a **l’annex 2**, *C. edulis* també es beneficia d’estructures mortes (el que hem anomenat herència física) per una vida millor dels successors o, fins i tot, del mateix material genètic, ja que afavoreix el creixement dels ramets vegetatius propis, allargant així la supervivència del genet en el temps. Ara bé, l’edat del genet és de difícil mesura, ja que com moltes espècies clonals, *C. edulis* podria no guardar una relació directa entre l’edat i la mida (De Witte i Stöcklin, 2010). La longevitat en espècies clonals s’ha descrit, per exemple, fins a 450 anys en l’espècie *Geum reptans* a través de l’estimació basada en models demogràfics als Alps suïssos (De Witte et al., 2011) i fins a milers d’anys en arbustos clonals o 10.000 anys en alguns arbres clonals (De Witte i Stöcklin, 2010).

La senescència pot contribuir a la supervivència d’una espècie clonal a tres nivells, a nivell foliar (òrgan), a nivell de ramet i a nivell de genet. En el cas de *C. edulis*, la senescència contribueix de forma directa al seu impacte sobre les propietats fisicoquímiques del sòl i sobre la reducció de la biodiversitat d’espècies natives. Cuddington i Hastings (2004) troben que les invasions poden ser especialment ràpides quan els organismes són capaços d’envair ambients subòptims però modificar-los per tal que esdevinguin més favorables. Aquest és el cas de la invasió de *C. edulis*, que malgrat l'aparent paradoxa, mor per viure més.

4. Retroalimentació positiva en la invasió de *C. edulis*

Tal com comentàvem, l'observació de Cuddington i Hastings (2004) destaca la modificació ecosistèmica per a la invasió ràpida i amb elevats impactes. Aquesta dinàmica porta a una modificació de l'ecosistema que facilita la pròpia invasió, promovent que sigui més ràpida. La recerca desenvolupada amb l'estudi de cas de *C. edulis* ens porta a descriure la retroalimentació positiva de la invasió de *C. edulis*, promovent la persistència i expansió de l'espècie.

L'estratègia dels cicles de creixement i mort (*Grow and die strategy*) presentada a **l'annex 2** estableix les bases d'aquest cicle, però els diferents estudis realitzats ens permeten entendre alguns dels mecanismes que contribueixen al fet que la invasió esdevingui una retroalimentació positiva. En el **capítol 2** veiem com el major esforç reproductor es relaciona amb una major capacitat de recuperació a l'estrès hídric estival i es proposa aquesta idea de la relació entre creixement sexual i asexual que contribueixen a l'expansió i persistència de l'espècie en millorar les condicions microclimàtiques. L'elevat esforç reproductor porta a una major producció de llavors que permetrà un major reclutament i persistència gràcies a l'alta resistència de les llavors (**annex 3**) i la seva longevitat, donant lloc a bancs de llavors persistents (**capítol 3**). Ara bé, aquest major esforç reproductor també permet l'acumulació de més matèria orgànica al sòl, facilitant les condicions per a les següents generacions (**annex 2**), fet que porta a un encara major esforç reproductor i promou així una dinàmica de retroalimentació positiva (**Figura 5**).

Per altra banda, unes millors condicions ambientals en individus amb elevat esforç reproductor es relacionen amb la millor recuperació a l'estrès hídric estival a nivell dels ramets vegetatius (**capítol 2**), fet que facilitaria la supervivència dels ramets a l'estrès sever i, per tant, contribuiria també al creixement de l'espècie i a la seva expansió. En reproduir-se, aquests ramets contribuirien també a millorar les condicions microambientals. L'elevada capacitat de fer front a l'estrès observada en els **capítols 1, 2, 4 i 5** contribuiria a aquest èxit asexual que també participaria a la retroalimentació positiva de la invasió de *C. edulis*.

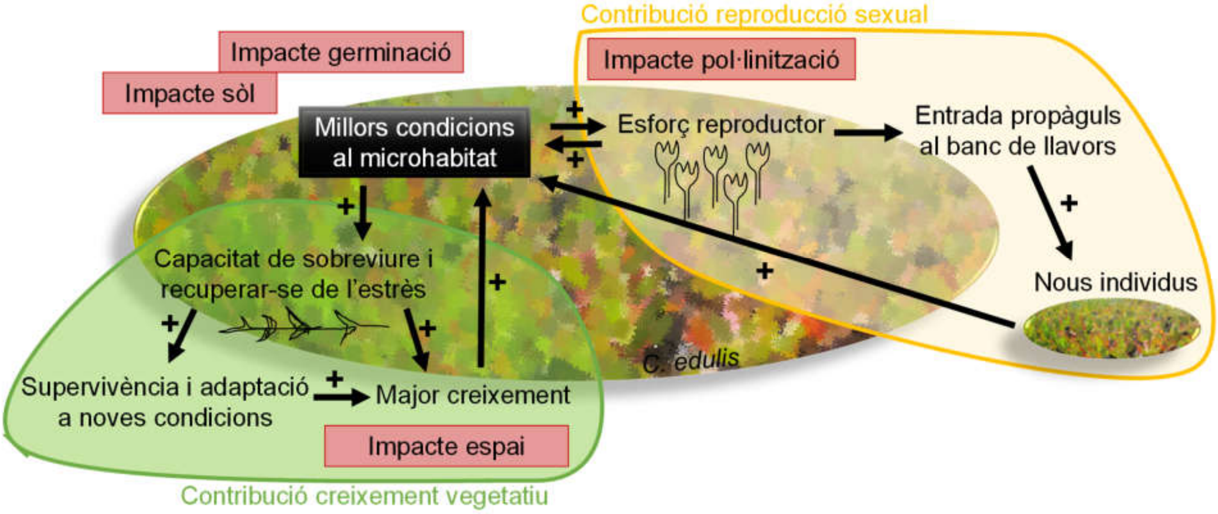

Figura 5. La retroalimentació positiva en la invasió de *C. edulis*. Contribució de la reproducció sexual i el creixement vegetatiu sobre l'èxit en la invasió de l'espècie, juntament amb els impactes.

La retroalimentació positiva en la invasió per *C. edulis* té també implicacions sobre l'impacte generat. Els impactes podrien augmentar també de forma no lineal amb la progressió de la invasió (**Figura 5**). Per una banda, la modificació de les propietats fisicoquímiques del sòl serà més extensiva amb l'expansió de l'espècie i més profunda, acidificant el sòl i dificultant les tasques de restauració del sòl un cop erradicada l'espècie. Els impactes sobre les espècies natives augmenten també en retroalimentació positiva. La retroalimentació promou l'alteració de les propietats del sòl, ja que impedeix la germinació d'espècies natives i redueix l'espai disponible per a altres espècies. També s'ha descrit que el pol·len de *C. edulis* pot afectar negativament a la producció de llavors l'espècie nativa *Helichrysum stoechas* a Mallorca (Jakobsson et al., 2008). A mesura que avança la invasió, es promouria l'esforç reproductor, fet que augmentaria la interferència, així com la competició per pol·linitzadors.

El responsable d'un increment no lineal dels costos d'erradicació no és només la retroalimentació en l'expansió de l'espècie, sinó que també hi incideix la retroalimentació en els impactes. Això s'explica per la major presència de l'espècie tant en superfície com al banc de llavors i pel grau d'alteració de l'ecosistema, fet que multiplica les dificultats de la seva gestió.

5. Components de la variabilitat en la invasió

5.1. Els components de la variabilitat

La variabilitat és soroll en l'estudi de la resposta individual i en els patrons de resposta i dinàmiques poblacionals i sol ser poc desitjable a nivell experimental. Aquesta variabilitat apareix a tots els nivells: des de les cèl·lules a les poblacions. Els estudis realitzats amb *C. edulis* han permès descriure la variabilitat de l'espècie a diferents nivells, els quals ens permeten distingir entre: plasticitat fisiològica, variabilitat intraindividual, variabilitat interindividual i variabilitat interpoblacional, que contribueixen a la invasió de *C. edulis* (**Figura 6**):

- **Variabilitat o plasticitat fisiològica:** En termes fisiològics, tal com es revisa a **l'annex 4,** la plasticitat és l'habilitat d'aclimatar-se, i possiblement adaptar-se, a diferents condicions ambientals. La plasticitat fenotípica, sobre la qual s'ha fet molta recerca, es defineix com la capacitat d'un genotip de produir diferents fenotips (Richards, 2006). Ara bé, la seva mesura i el seu significat són molt complexes. La **plasticitat fisiològica** faria referència a la variabilitat a conseqüència d'una variació en les condicions ambientals, que provoca la necessitat d'un ajust fisiològic en un genotip. Diversos estudis han posat en relleu la capacitat de les espècies mediterrànies d'ajustar la seva morfologia, fisiologia, fenologia i reproducció en resposta als canvis en la temperatura, llum, disponibilitat hídrica i nutrients (Domínguez et al., 2012; Matesanz i Valladares, 2014). Sota el concepte de 'plasticitat antioxidant' entenem el conjunt de canvis quantitatius en el perfil dels metabòlits antioxidants que observem en resposta a un estrès ambiental (Fernández-Marin et al., 2017; Esteban et al., 2015). La revisió de les estratègies de fotoprotecció de 73 espècies mediterrànies revela que, malgrat que no presenten compostos fotoprotectors diferents a la flora d'altres regions, les espècies mediterrànies mostren majors índexs de plasticitat, fet que podria beneficiar-les en el marc del canvi climàtic global (Fernández-Marin et al., 2017). En concordança amb aquests resultats, en els diferents capítols s'ha destacat la gran plasticitat de *C. edulis* en les seves respostes fisiològiques als gradients ambientals estudiats. En el **capítol 1** veiem la plasticitat fisiològica de l'espècie en l'estratègia fotoprotectora durant l'any en condicions naturals, que mostra una resposta més plàstica que una nativa amb què coexisteix. En el **capítol 2** veiem una gran plasticitat en respondre a un intens període amb baixa disponibilitat hídrica en condicions naturals i la seva posterior recuperació. Finalment, en el **capítol 4** veiem plasticitat en la resposta hormonal a les baixes temperatures, en funció de la severitat i duració de l'estrès.

- **La variabilitat intraindividual**: A nivell d'un individu, un mateix tipus de teixit pot presentar grans diferències sota unes mateixes condicions ambientals degut a factors extrínsecs tals com gradients ambientals, o a factors intrínsecs com diferències en el desenvolupament o cavis epigenètics (Herrera, 2017). Quan aquestes diferències ocorren entre fulles, parlem d'heterofília, entre fruits, heterocàrpia, i entre llavors, heteromorfisme. Aquesta variabilitat té un rol adaptatiu que permet ajustar-se a l'heterogeneïtat ambiental (Nakayama et al., 2017). En el cas de *C. edulis*, durant l'estiu les fulles de ramets diferents presenten grans diferències en funció de la presència de càrrega reproductora. Hi ha una elevada variabilitat intraindividual que es manifesta en l'eficiència fotosintètica, en els continguts de pigments fotosintètics, carotenoides i la seva de-epoxidació, en el contingut d'α-tocoferol, en continguts hormonals i en l'acumulació de productes derivats de l'oxidació lipídica (**capítols 1 i 2**). A més, responen de forma diferencial a les condicions ambientals, ja que mostren una major sensibilitat al punt de màxim estrès al **capítol 1,** i vies de senyalització hormonals diferents per recuperar-se de la sequera al **capítol 2**.

- **Variabilitat interindividual**: equival a l'amplitud de canvi entre els diferents genotips sota unes mateixes condicions macroambientals. A banda del genotip, les condicions microambientals, els processos de memòria o l'estadi de desenvolupament participen en augmentar la variabilitat interindividual. En *C. edulis* s'ha observat una gran variabilitat interindividual, tant en controlar les condicions ambientals com en condicions naturals, tal com hem observat en els **capítols 2, 4** i **5,** en què s'ha controlat el factor individu. En condicions controlades (**capítols 4 i 5**), la variabilitat entre els diferents individus (apreciable en l'error estàndard) era, en general, menor que en condicions naturals (**capítol 2**). Això és degut a la gran quantitat de factors operen en condicions naturals tals com els gradients ambientals en el sòl o en la llum rebuda, la presència d'altres espècies, etc., fet que s'ha ajustat amb un major nombre de rèpliques en condicions naturals. Un dels casos més visibles de variabilitat interindividual observada és el rang d'esforç reproductor de les mates de *C. edulis* estudiades en el **capítol 2**, que mostraven valors des de 13 a 153 fruits/m^2.

- **Variabilitat interpoblacional o geogràfica**: la definim com la variabilitat entre poblacions separades geogràficament de manera que combina la variabilitat en el conjunt de genotips i l'ambient. Aquesta variabilitat es va considerar en el **capítol 1** amb el factor 'Zona', que distingia tres zones diferenciades ubicades entre 100 i 200 m, però no vam trobar diferències significatives sobre cap paràmetre amb relació a les respostes fotoprotectores i la seva variació anual. Per contra, en el **capítol 3** s'observa un fort efecte geogràfic entre poblacions que es troben entre 300 m i 3,4 km en paràmetres relacionats amb la reproducció sexual. Els resultats, per tant, suggeririen un contrast en la variabilitat geogràfica en diferents paràmetres, essent molt més variables els atributs relacionats amb la reproducció sexual tals com la producció de llavors, que les respostes fisiològiques a l'estrès. En suport d'aquesta hipòtesi, l'estudi de Bartomeus i Vilà (2009) va explorar la producció de llavors de *C. edulis* i *Opuntia stricta* en poblacions ubicades també a 3 km de distància, revelant variabilitat geogràfica en alguns dels tractaments de pol·linització aplicats sobre la primera, fet que suggereix que la variabilitat podria ser una interacció diferencial amb pol·linitzadors. Malgrat que això contribuiria a explicar la major variabilitat en els atributs relacionats amb la reproducció sexual, cal posar damunt la taula la localització de la població analitzada i l'efecte geogràfic: els resultats del **capítol 3** apunten que la zona considerada en el **capítol 1** (ubicada al Cap de Creus) seria la que tindria menys variabilitat geogràfica d'entre les tres comparades (Cap de Creus, Costa Brava i Maresme), fet que s'atribueix a la baixa presència humana. A més, l'efecte geogràfic considerat va ser més gran en el **capítol 3** (poblacions situades entre 800 m i 1,5 km al Cap de Creus) que en el **capítol 1** (zones ubicades entre 100 i 200 m). La diferència entre els individus del rang natiu i del rang invasor de *C. edulis* en el **capítol 5** també forma part d'aquesta variabilitat interpoblacional, on els individus del rang invasor varien funcionalment en mostrar una major tolerància a les baixes temperatures.

Malgrat que les diferents definicions dels components de la variabilitat són clares, a nivell pràctic, la seva mesura pot ser complexa. Per exemple, la variabilitat associada a la mesura de la variable que comparem és inevitable. Associada a aquesta variabilitat metodològica, l'ús de mètodes destructius impliquen que mai podrem mesurar la mateixa fulla en condicions ambientals diferents, de manera que la mesura de la plasticitat fisiològica podria estar incloent variabilitat intraindividual, o fins i tot intraorgànica (variabilitat entre la part de l'òrgan mesurat, àpex o base de la fulla per exemple). Les variacions microambientals també ens dificulten poder aïllar els diferents components de la variabilitat, especialment en condicions naturals. Encara és més problemàtica la distinció entre individus diferents que permetrien delimitar la variació interindividual, especialment en espècies clonals.

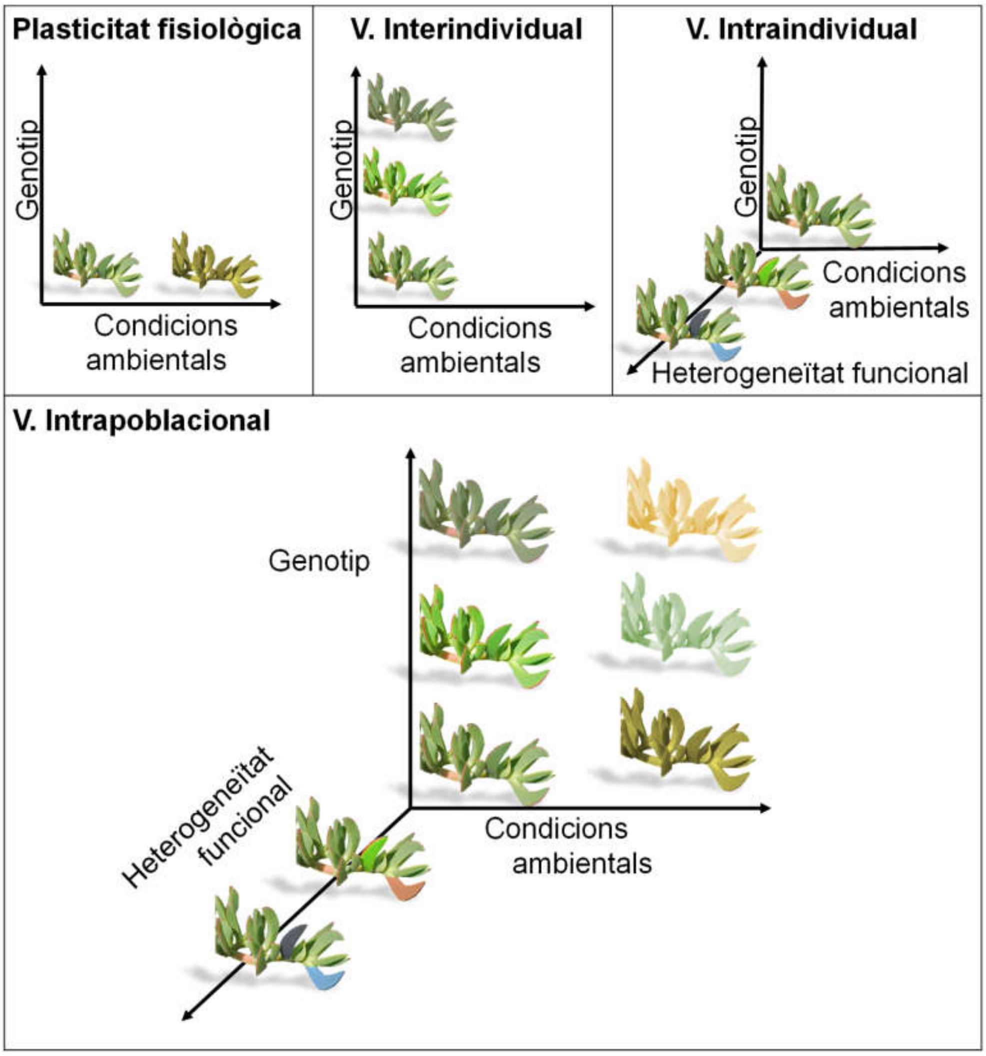

Figura 6. Components de la variabilitat en *C. edulis*, disposades en tres eixos referents a les condicions ambientals, el genotip i l'heterogeneïtat funcional. V: Variabilitat.

5.2. La variabilitat en la invasió

Els diferents components de la variabilitat identificats en *C. edulis* contribueixen de forma diferencial en el seu èxit invasor. Per exemple, la plasticitat fisiològica permet a les espècies invasores la colonització de nous ambients a través de l'ajust fisiològic adequat per sobreviure a unes noves condicions (Richards et al., 2006). La plasticitat proveeix d'un reservori per fer front a canvis climàtics ràpids i assistir l'adaptació (Lande, 2009; Chevin et al., 2010). De forma teòrica, una major plasticitat permetria a una espècie invasora colonitzar més quantitat d'hàbitats diferents, sobreviure a pertorbacions o episodis climàtics extrems (Valladares et al., 2006; Bennett et al., 2016). L'estudi de Higgins i Richardson (2014) demostra que les plantes invasores tenen una amplitud fisiològica del nínxol major que les natives. Funk (2008) també planteja que la plasticitat en atributs fisiològics i morfològics d'espècies invasores pot proveir un major accés a recursos limitats i afavorir el seu èxit. Malgrat aquests resultats, també n'hi ha altres que apunten al fet que els individus natius presenten una major plasticitat que individus invasors (Kleine et al., 2017) o plantegen que la falta d'enemics naturals alliberaria la pressió de selecció i l'evolució portaria a la fixació de genotips (Huang et al., 2015). També s'ha plantejat que la plasticitat pot no ser adaptativa, ja que pot estar reflectint sensibilitat a un factor ambiental. La plasticitat beneficiosa serà aquella que proporcioni homeòstasi en l'eficiència biològica de l'individu i, per tant, caldria connectar els ajustos fisiològics als canvis en l'eficàcia biològica. Ara bé, tal com es discuteix a **l'annex 4**, la connexió entre la plasticitat i l'eficàcia biològica no és sempre directa i pot variar en funció de l'escala que considerem o els canvis en el futur ambient. Tot plegat forma part d'un debat encara viu sobre els costos, límits i adaptabilitat de la plasticitat, en el qual les espècies invasores poden ser claus per entendre l'adaptació de les espècies a nous escenaris climàtics tals com l'escalfament global (Nicotra et al., 2010; Valladares et al., 2014).

La plasticitat fisiològica a nivell intraindividual participa en l'adaptació i l'expansió a l'hàbitat. En *Persea americana* s'ha observat una elevada variabilitat en la resistència del xilema als embolismes entre diferents fulles d'un mateix arbre, que determina patrons temporals i espacials marcats de senescència foliar, i permeten a l'espècie sobreviure a un fort estrès hídric prioritzant les fulles més productives (Cardoso et al., 2020). L'estudi de la variabilitat intraindividual amb *Helleborus foetidus* revela que hi ha relació entre la fecunditat i la variabilitat entre la mida de les fulles, pes foliar específic i atributs estomàtics d'un individu, que li permetrien optimitzar l'eficiència en l'ús de l'aigua enfront de la variabilitat ambiental (Herrera et al., 2015; Herrera, 2017). Un atribut molt comú d'espècies clonals és la integració fisiològica entre ramets que permet la divisió de tasques i especialització. La divisió de tasques descrita en *C. edulis* (Roiloa et al., 2013; Roiloa, 2019), juntament amb la variabilitat intraindividual descrita als **capítols 1 i 2**, contribueix a definir el conjunt de l'individu com un 'mosaic funcional', amb l'objectiu d'afrontar l'heterogeneïtat de l'espai, optimitzant al màxim els recursos disponibles. Així doncs, la variabilitat intraindividual és especialment beneficiosa per a l'expansió vegetativa d'espècies clonals en hàbitats heterogenis.

La variabilitat interindividual és clau per a l'adaptació, ja que és el mecanisme principal sobre el qual actuen els processos evolutius i constitueix un reservori de possibilitats. La variabilitat interindividual és la base de l'efecte fundador i el motor dels processos d'evolució ràpida en el rang invasor. Els individus invasors de *Lythrum salicaria* presenten una major tolerància biòtica genèticament determinada en comparació amb els natius, fet que ha contribuït en el seu èxit invasor (Joshi i Tielbörger, 2012). Sense variabilitat interindividual aquest procés d'evolució ràpida no hauria estat possible, de la mateixa manera que l'expansió d'aquesta espècie no hauria estat possible sense la plasticitat d'aquesta en el temps de floració (Colautti i Barrett, 2013). Resultats similars també s'han observat en *Ambrosia artemisifolia* en la seva invasió a Xina, on processos d'evolució ràpida han portat al genotip invasor a majors taxes de creixement i a major plasticitat (Sun i Roderick, 2019). En el cas d'*Helianthus tuberosus*, la invasió també ha estat possible gràcies a la plasticitat en diferents atributs entre els individus (variabilitat interindividual), que a través de l'acomodament genètic s'han fixat a la població invasora (Bock et al., 2018). Malgrat que en el cas de *C. edulis* no disposem de l'anàlisi genètica, la diferenciació sota unes mateixes condicions que observem al **capítol 5** donaria suport a la hipòtesi que hi ha hagut un procés d'evolució ràpida en individus del rang invasor. Aquest procés d'evolució ha estat clau en l'expansió de l'espècie al continent Europeu.

Finalment, la variabilitat interpoblacional contribueix en la invasió, ja que reflecteix la capacitat d'adaptació local. Per exemple, en diferents espècies s'han descrit ajustos adaptatius als ambients locals mediterranis en uns gradients ambientals en la mida de la llavor (Metz et al., 2010). La variabilitat interpoblacional combina l'efecte de la variabilitat en les condicions ambientals, però també l'efecte genètic, el qual pot ser degut a diferències en el material genètic de partida o bé conseqüència de l'evolució ràpida associada a l'adaptació local. Alguns estudis han descrit que les espècies invasores tindrien una variació genètica més reduïda en comparació al seu rang natiu (Dlougosh i Parker, 2008). Ara bé, l'estudi recent desenvolupat per Smith et al. (2020) a escala global amb la invasora clonal *Plantago lanceolata* ha revelat que les múltiples introduccions al rang invasor han promogut una elevada diversitat genètica homogènia en el territori. Per contra, les regions natives mostren una forta estructura genètica en l'espai. En el cas de *Lythrum salicaria,* també s'ha demostrat que múltiples introduccions han promogut la barreja genètica en diferents fronts d'invasió als Estats Units (Shi et al., 2018). L'associació de *C. edulis* amb l'home per les seves característiques com a fixadora dunar i planta ornamental podrien haver promogut múltiples introduccions al territori, així com una elevada pressió de propàgul. En aquest sentit, l'anàlisi del **capítol 3** revela que les zones amb més variabilitat geogràfica són les zones amb major presència humana. La presència humana, relacionada amb la degradació dels hàbitats, contribueix a explicar la riquesa d'espècies exòtiques, posant de manifest que la disminució de la resistència biòtica facilita la invasió d'espècies exòtiques tal com prediu la hipòtesi de la resistència biòtica (Bartomeus et al., 2012). La manca de gradients de variació amb la distància geogràfica en el **capítol 3** podrien suggerir que la variabilitat geogràfica és deguda a l'adaptació local.

La plasticitat fisiològica, la variabilitat intraindividual, la variabilitat interindividual i la variabilitat interpoblacional són un reservori de possibilitats per a l'adaptació i, per tant, resulten claus en l'èxit de les poblacions invasores. Fins i tot a una escala superior, la biodiversitat o diversitat d'espècies confereix estabilitat als ecosistemes, malgrat que aquesta sí que ens interessa preservar-la.

6. Implicacions per la gestió d’espècies invasores

La problemàtica de les espècies invasores s’accentua per la manca d’estratègies rendibles per tal d’eliminar, mitigar o prevenir els seus impactes. Aquestes estratègies poden operar en les diferents fases de la invasió: transport, introducció, establiment i dispersió. La gestió de l’impacte de les espècies invasores la podrem fer a través d’entendre els seus mecanismes, establir polítiques de bioseguretat, mitigar els seus impactes amb xarxes de vigilància o eliminar els impactes existents erradicant l’espècie i duent a terme activitats de restauració dels hàbitats. Alguns estudis demostren la discordança entre la recerca sobre espècies invasores i la informació sobre la seva gestió, apuntant que hi ha un gran buit conceptual (Matzek et al., 2015). Funk et al. (2020) recentment destaquen les diferents àrees de l’ecologia de les invasions que són importants per a la gestió: els bancs de llavors, mecanismes de dispersió i expansió, cicle de vida, impactes, canvi climàtic i successió ecològica.

El conjunt d’estudis realitzats sobre *C. edulis* té implicacions que podrien ser generalitzables en la gestió en les diferents fases de la invasió, especialment pel que fa a entendre els mecanismes de la invasió, la prevenció, la dispersió i l’erradicació d’espècies invasores.

6.1. Què porta a una espècie a ser invasora? Què hem après de *C. edulis*?

Tal com he presentat a la introducció, hi ha diverses hipòtesis que pretenen explicar per quin motiu algunes espècies exòtiques esdevenen invasores. Malgrat que algunes han estat demostrades en algunes espècies, fallen en explicar l’èxit d’altres espècies reflectint que encara queda molta recerca per fer en el camp de la biologia de les invasions (Heger et al., 2013). En el cas de *C. edulis*, els factors que la porten a ser una invasora exitosa al Mediterrani són els següents:

a) Gran plasticitat en resposta a gradients ambientals.

b) Atributs clonals, elevada inversió en reproducció sexual, desenvolupament d'un banc de llavors permanent i un cicle de vida curt (a nivell de ramet).
c) Retroalimentació durant la invasió a través de la modificació de les condicions ambientals per a fer-les més favorables, augmentant el seu impacte.
d) Elevada pressió de propàgul pel seu interès ornamental però també funcional com a fixadora dunar.
e) Facilitat per colonitzar ambients pertorbats o urbans que n'afavoreixen la variabilitat.

D'aquest llistat és important destacar que, per una banda, hi ha factors intrínsecs de l'espècie que la fan una gran candidata a invasora segons la hipòtesis de la invasora ideal, com podrien ser els atributs clonals, elevada producció de propàguls o la plasticitat envers gradients ambientals que s'han descrit com a beneficiosos per a la invasió (Bossdorf et al., 2005; Richardson i Pyšek, 2006; Pyšek i Richardson, 2007; Pyšek et al., 2009, 2015; van Kleunen et al., 2010; Moravcová et al., 2015; Gioria i Pyšek, 2016), però per altra banda, també trobem com l'home i l'hàbitat a envair té també un rol determinant en l'èxit de l'espècie promovent una alta pressió de propàgul i degradant ecosistemes que en facilitarien l'èxit.

El paper de l'home en el transport d'espècies invasores és evident: l'augment en el nombre d'espècies invasores arreu del món ha anat acompanyat del desenvolupament tecnològic del trànsit per terra, aire i mar i també l'augment de les relacions comercials entre diferents regions del món. Països amb elevats recursos econòmics hostegen també 30 vegades més espècies invasores en comparació a països de baix poder adquisitiu (Seebens et al., 2018). La introducció d'espècies exòtiques fora el seu rang natiu es pot categoritzar com intencionada, o bé no intencionada. Les introduccions intencionades poden acompanyar accions nobles com aportar noves espècies per la fixació de talussos derivats de la construcció d'infraestructures, el disseny de cobertes verdes amb espècies resistents o la reducció de la petjada ecològica de la importació de productes agroalimentaris. També poden acompanyar interessos comercials per a l'explotació de l'espècie, com el cas d'espècies amb interès silvícola o perseguir finalitats recreatives, com el cas de grans peixos per a la pesca esportiva al Delta de l'Ebre. En les invasions vegetals, la jardineria ha jugat un paper molt important com a via d'introducció intencionada, aportant gran diversitat exòtica. De fet, un 48 % de la flora exòtica d'Espanya ha entrat a través de la jardineria, la qual suposa la via d'introducció majoritària, doblant la segona major causa que és l'agricultura (18 %), seguida de les introduccions involuntàries (16 %) (Sanz Elorza et al., 2004). Més recentment, l'estudi desenvolupat per Bayón i Vilà (2019) exposa que d'entre les 914 espècies exteriors que es venen a Espanya, un 77% són exòtiques i en alguns casos presenten un elevat risc d'invasió. A escala mundial, entre un 75 i un 93 % de la flora exòtica naturalitzada prové de jardins botànics o domèstics (van Kleunen et al., 2018). Entre aquests percentatges hi trobem *C. edulis*, una espècie d'interès ornamental i que, per tant, ha gaudit d'una pressió de propàgul gran en el seu rang invasor. El paper de l'home en augmentar la pressió de propàgul de les plantes ornamentals és especialment important i s'ha descrit que l'actual conjunt de flora exòtica europea d'interès ornamental constitueix un deute d'invasió en el context del canvi climàtic (Haeuser et al., 2018). No només la introducció inicial juga un paper clau en la probabilitat que una espècie esdevingui invasora. Les introduccions múltiples augmentarien la pressió de propàgul i afavoririen la diversitat genètica, fins i tot per sobre de la diversitat genètica del rang natiu (Smith et al., 2020).

A banda del paper de l'home en la pressió de propàgul, la destrucció i degradació d'hàbitats també contribueix a afavorir l'entrada i l'impacte de les invasions (Elton, 1958; Hobbs i Huenneke, 1992). Els ambients degradats podrien ser més fàcilment envaïts (hipòtesi del règim de pertorbacions) per l'elevada disponibilitat de recursos (hipòtesi de l'elevada disponibilitat de recursos), per la presència de nínxols buits (hipòtesi dels nínxols buits) o bé per la menor resistència biòtica (hipòtesi de la resistència biòtica) (Lear et al., 2020). Independentment de l'espècie, aquestes hipòtesis presenten que els hàbitats degradats serien més fàcilment envaïts per espècies exòtiques. En el cas de *Alternanthera philoxeroides*, la seva capacitat de tolerar ambients degradats li permetria disposar d'un avantatge competitiu sobre espècies natives coexistents (Wang et al., 2018). Northfield et al., (2018) destaquen que algunes espècies natives podrien actuar com a facilitadores del procés d'invasió i presenta la denominació de natius renegats (*native turncoats*) que facilitarien la invasió a través de relacions de mutualisme, cascada tròfica i depredació d'un competidor, entre altres mecanismes.

Així doncs, considerant els estudis realitzats en *C. edulis*, no és només l'espècie, sinó també les condicions de la seva invasió, que podrien per igual determinar que una espècie esdevingui o no invasora (**Figura 7**). Semblaria, doncs, que l'èxit de les invasions no depenen únicament de l'espècie en qüestió, sinó que també, i de manera molt important, depenen de factors humans més enllà del seu transport. En aquest sentit, la revisió dels mecanismes de la invasió realitzada per Sinclair et al. (2020) presenta com a crítics els atributs i comportaments dels vectors de transport lligats a l'home per a l'èxit de les invasions tant per l'entrada, la supervivència i èxit de les espècies invasores. Així doncs, la problemàtica de les invasores va més enllà del seu impacte degut a la interacció amb la societat.

L'article recent d'Enders et al., (2020) que dibuixa el mapa conceptual de les hipòtesis de la invasió, planteja que l'actual xarxa d'hipòtesis no inclou de forma dominant la importància dels humans com a conductors de les invasions i preveu que aquesta visió canviï en els propers anys. Kueffer (2017) també planteja que el camp de la invasió biològica doni un gir entorn aquest rol dels humans en els propers anys.

Figura 7. Factors que porten a una espècie a ser invasora en funció de la reflexió sobre la invasió de *C. edulis* al Mediterrani.

6.2. Implicacions en la prevenció de l'entrada i la dispersió

La recerca desenvolupada *sobre C. edulis* posa de manifest la importància de la prevenció en l'entrada d'espècies invasores, ja que la dificultat en la seva gestió i la magnitud dels seus impactes incrementa de forma exponencial durant la progressió de la seva invasió. Prevenir l'establiment d'espècies exòtiques és l'eina amb major rendibilitat. Donat que generalment el control o l'erradicació d'espècies invasores és difícil i molt costós, la prevenció hauria de ser el recurs principal en la lluita contra les invasions biològiques (Hulme, 2006). Actualment, les accions de prevenció d'espècies exòtiques invasores a Espanya es basen en la prohibició de la importació, venda, cultiu, ús i alliberament al medi d'espècies catalogades, juntament amb la realització de campanyes de sensibilització i divulgació als ciutadans en centres educatius i culturals (Reial Decret 630/2013). Segons la Campanya per a la prevenció, minimització i eliminació de riscos d'Espècies Exòtiques Invasores, impulsada per la Federació Espanyola de Municipis i Províncies en col·laboració amb el Ministeri d'Agricultura, Alimentació i Pesca, els missatges prioritaris en la sensibilització són: evitar abandonaments i el comerç il·legal i transmetre a la ciutadania els possibles problemes sanitaris i l'alt cost de control i gestió que suposen les espècies exòtiques invasores. El passat any, es va publicar el Reial Decret 216/2019, de 29 de març, pel qual s'aprova la llista d'espècies exòtiques invasores preocupants per a la regió ultraperifèrica de les illes Canàries i pel que es modifica el Reial Decret 630/2013, de 2 d'agost, pel que es regula el Catàleg espanyol d'espècies exòtiques invasores. Malgrat que el llistat d'espècies preocupants de moment sigui només per a les illes Canàries, és un avenç significatiu, ja que suposa que no només es restringeixen les espècies exòtiques que ja estan causant un impacte, sinó que s'apliquen restriccions a espècies que podrien causar-lo. Ara bé, la redacció d'aquesta llista per a un territori més ampli, com és tot l'estat Espanyol, suposa una tasca d'elevada complexitat tal i com exposa l'estudi de Bayón i Vilà (2019) que classifica en sis llistes les espècies ornamentals en funció del seu risc invasor i destaca que pel 61% de les espècies exòtiques ornamentals no es disposa de dades suficients per a l'anàlisi de risc.

La dificultat de definir quines espècies podrien causar impacte si arribessin a un territori s'exposa en el **capítol 5**. La similitud climàtica s'utilitza com a criteri de delimitació d'espècies exòtiques, sota el supòsit que encara que permetem que les espècies saltin el filtre geogràfic, pot no superar el filtre abiòtic. Ara bé, aquesta suposició, malgrat que ha estat recolzada en alguns estudis (Pearman et al., 2008; Petitpierre et al., 2012; Guisan et al., 2014), també s'ha posat en dubte en altres (Broennimann et al., 2007; Gallagher et al., 2010; Early i Sax, 2014). En analitzar canvis en el nínxol climàtic d'espècies introduïdes, entre un 65-100 % de 815 plantes terrestres introduïdes als cinc continents van mostrar canvis en el nínxol climàtic (Atwater et al., 2018). La conservació del nínxol climàtic s'ha descartat en el cas de la invasió de *C. edulis* a Europa (**capítol 5**), ja que la presència de l'espècie supera totalment les previsions basades en la similitud climàtica. Més d'un 90 % de la invasió a Europa era imprevisible. La previsió d'invasions per similitud climàtica del rang natiu és errònia, ja que diferents processos poden fer que la planta canviï donant lloc a canvis en el nínxol, permetent que ocupi llocs que serien totalment imprevistos. Els models de distribució d'espècies basats en espècies taxonòmicament properes tampoc són útils per preveure la distribució d'una altra (Aguirre-Gutiérrez et al., 2015). Els canvis evolutius ràpids en el rang invasor han estat demostrats en diverses espècies invasores, i s'han suggerit en altres estudis de *C. edulis* respecte la capacitat de divisió de tasques (Roiloa et al., 2016). Aquests canvis poden ser deguts a processos de deriva genètica en les poblacions fundadores, hibridació amb altres espècies o bé l'aparició de noves pressions de selecció (Bossdorf et al., 2005). La velocitat de l'expansió en modelitzar escenaris d'evolució d'espècies invasores augmenta considerablement (Phillips, 2015). Així doncs, la definició d'hàbitats que podrien ser envaïts per preveure futures invasions ha de contemplar la capacitat de canvi (Phillips, 2015), i/o incloure l'anàlisi del nínxol fonamental de l'espècie, construint així models de distribució d'espècies mecanicistes (Kearney i Porter, 2009; Evans et al., 2015; Chapman et al., 2017).

Per a la delimitació d'espècies amb risc d'invasió també és important tenir en compte que les prediccions de zones que podrien ser envaïdes i del comportament invasor d'una espècie podrien ser errònies si no es considera la variabilitat geogràfica d'aquesta. En el **capítol 3** observem una gran variabilitat geogràfica en trets de les llavors de *C. edulis*, donant lloc a dinàmiques diferents en el banc de llavors. Aquest estudi revela la importància d'incorporar la variabilitat interindividual en els SDM per preveure futures invasions.

Finalment, una altra dificultat en l'elaboració d'un llistat d'espècies amb risc d'invasió són les condicions canviants que poden afectar la definició d'hàbitats a envair per part de les espècies exòtiques. En el marc l'escalfament global, per exemple, tant l'elevada plasticitat de *C. edulis* a l'estrès hídric observada en el **capítol 2** com la delimitació d'àrees potencialment adequades a Europa en el **capítol 5,** suggereix que un augment de la sequera i les temperatures podria beneficiar l'espècie al Mediterrani i a latituds superiors respectivament.

Degut a les dificultats de preveure quines espècies exòtiques podrien esdevenir invasores, és especialment important evitar la dispersió d'espècies exòtiques en el territori. En aquest sentit, amb l'objectiu de mitigar la dispersió i expansió d'espècies exòtiques un cop introduïdes i prevenir els seus impactes, els resultats obtinguts en els **capítols 3 i 5** posen de manifest la importància del paper de l'home facilitant les invasions. Per una banda, en el **capítol 3** veiem com la degradació i fragmentació dels ecosistemes associada a la presència humana promouen la variabilitat en els atributs relacionats amb la reproducció sexual de l'espècie, fomentant la invasió. Per altra banda, els resultats del **capítol 5** plantegen el paper de la pressió de propàgul fora de les condicions òptimes de l'espècie per l'adaptació i expansió de *C. edulis*. En aquest sentit, s'exposa el perill de les plantes ornamentals: mantenir-les fora les seves condicions pot promoure l'adaptació. En el cas d'*Helianthus tuberosus*, el seu cultiu fora el seu rang de distribució natural i l'aplicació de programes de millora de cultius han promogut variació genètica que ha afavorit el seu comportament invasor i actualment és una espècie invasora en múltiples regions d'Europa (Bock et al., 2018). Malgrat que algunes plantes exòtiques es mantinguin en vivers i lluny d'hàbitats naturals disponibles (per exemple en les ciutats), la venda i distribució d'aquestes espècies augmenta molt la pressió de propàgul tant d'entrada com de dispersió i és necessari aplicar polítiques específiques adreçades a plantes exòtiques ornamentals (Hulme et al., 2017). Tal com s'ha comentat anteriorment, en el problema de les invasions, l'home no només juga un paper clau en el transport d'espècies fora el seu rang natiu, sinó que també participa de forma directa (cuidant espècies fora les seves condicions òptimes) o indirecta, ja que els ambients urbans faciliten la invasió.

Per tant, l'estudi de *C. edulis* ens permet inferir que la prevenció de la dispersió i expansió de les espècies invasores, la divulgació de la perillositat d'aquestes, la consciència ambiental, la preservació dels hàbitats i la reducció de l'impacte de les estructures de la tecnosfera juguen un paper clau en la gestió de la problemàtica de les espècies invasores, que requereix actuacions transversals sobre tots aquests factors.

6.3. Implicacions en l'erradicació

L'erradicació definitiva d'una espècie invasora només acostuma a ser viable durant les primeres fases d'introducció o en poblacions aïllades (Myers et al., 2000, Zavaleta et al., 2001; Groves i Panetta, 2002). Degut als creixents costos, les estratègies de gestió han de procurar la màxima rendibilitat. Actualment, la gestió enfocada en l'erradicació d'espècies invasores es basa en l'aplicació d'algun o més d'un d'aquests mètodes de contenció i control: manual, mecànic, químic o biològic.

Concretament, les actuacions per l'erradicació de *C. edulis* es centren en el tractament físic i/o químic (Guirado et al. 2017; Campoy et al., 2018). Per una banda, el tractament físic es fa per arrancada manual o amb maquinària petita per evitar danys i és el tractament més efectiu (Fraga et al., 2005). El tractament de les restes és important per evitar la reinvasió, ja que els ramets de *C. edulis* arrancats fàcilment podrien arrelar, fins i tot al cap de sis mesos d'emmagatzematge (Souza-Alonso et al., 2018). Desplaçar les restes no és recomanable, i és preferible apilar-les i cremar-les al cap d'un any i mig d'haver estat arrancades. La reducció del volum de la pila s'estima del 75 % al cap de dos anys (Guirado et al., 2017). És una actuació altament costosa i amb un risc molt elevat de reinvasió, de manera que es recomana seguiment a llarg termini almenys de deu anys (Ruffino et al., 2015). Experiències en tractament químic per erradicar *C. edulis* s'han basat en l'aplicació de glifosfat 2 % amb un 1 % de sulfactant o aigua acidificada durant l'hivern o durant la primavera. L'ús de dues aplicacions de glifosfat, juntament amb revisió i extracció manual a la Toscana (Itàlia) en una àrea de 8.000 m^2, va resultar molt efectiva per erradicar l'espècie i recuperar la diversitat nativa al cap de pocs anys (Lazzaro et al., 2020). Malgrat l'eficàcia d'aquest tractament, el glifosfat pot persistir al sòl durant anys impedint la restauració ecosistèmica i el seu ús es desaconsella especialment a sòls sorrencs (Hueso Alcaide, 2017). Una alternativa a aquests tractaments és el control biològic. En aquest sentit, recentment s'han realitzat estudis explorant l'ús de tractaments biològics per la contenció de l'espècie amb l'herbívor especialista *Pulvinariella mesembryanthemi* o el fong *Sclerotinia sclerotiorum* (Vieites-Blanco et al., 2019; Vieites-Blanco et al., 2020).

Un dels problemes principals en la gestió de *C. edulis* és que requereix vigilància i accions a llarg termini degut a la seva capacitat de formar un banc de llavors permanent. L'estudi de Matzek et al. (2015) posa de manifest la discordança que hi ha entre la informació sobre els bancs de llavors d'espècies invasores que voldrien els gestors i la recerca publicada. En aquest sentit, els resultats de l'anàlisi de sensibilitat a diferents temperatures de llavors de *C. edulis* de **l'annex 3** suggereixen que tractaments de solarització podrien permetre l'erradicació del banc de llavors després de la retirada en sotmetre el sòl a elevades temperatures.

Per altra banda, degut a l'àmplia presència de l'espècie en el territori, és important prioritzar la gestió a les àrees que presenten major risc invasor o major impacte. En aquest sentit, els resultats obtinguts en el **capítol 2** i **l'annex 2** destaquen la importància de l'elevat esforç reproductor sobre l'expansió de l'espècie, però també sobre les respostes a l'estrès. Zones amb elevat esforç reproductor, així com l'elevada acumulació de matèria orgànica, podrien reflectir un estat avançat en la retroalimentació de *C. edulis*. Aquestes zones són, per tant, especialment perjudicials per a l'ecosistema, de manera que caldria prioritzar la seva gestió. Com a eina de priorització, també es destaca la descripció del mètode d'envelliment accelerat per caracteritzar la longevitat de les llavors de *C. edulis* descrit a **l'annex 3** que ofereix un mètode ràpid per comparar la durabilitat de les llavors. Juntament amb la caracterització de la producció de llavors, aquest test pot permetre prioritzar aquelles zones amb major probabilitat de creació d'un banc de llavors permanent tal com es descriu al **capítol 3**, que a llarg termini requeririen major inversió en seguiment i mesures de restauració.

Finalment, la delimitació d'hàbitats amb risc d'invasió pot ser també una eina útil per establir objectius prioritaris per minimitzar l'expansió a zones d'elevat valor biològic. En aquest sentit, descriure el nínxol fonamental de l'espècie ens permet projectar les zones d'hàbitat potencial de l'espècie en el territori i el canvi degut a diferents condicions ambientals com un augment de la desertificació degut al canvi climàtic (Thuiller et al., 2005). La construcció de SDM pot ser una eina útil per la delimitació de zones d'habitat potencial i projecció sota noves condicions ambientals, però considerant els resultats obtinguts en *C. edulis* al **capítol 5**, els SDM s'han de basar en anàlisis del nínxol ecològic i fonamental del genotip invasor, pel fet que pot haver patit efectes d'evolució ràpida que el diferenciïn del genotip natiu.

Conclusions

Conclusions

- L'espècie nativa *C. maritimum* i la invasora *C. edulis* presenten estratègies de fotoprotecció diferents, però ambdues eficients per superar l'estiu al Mediterrani. *C. edulis* presenta una major plasticitat anual.

- *C. edulis* presenta una gran plasticitat enfront de la baixa disponibilitat hídrica. Malgrat el procés de senescència associat a la reproducció, les fulles dels ramets reproductors són capaces de recuperar el seu contingut de clorofil·les després d'un estrès sever.

- L'esforç reproductor de *C. edulis* condiciona les respostes foliars a l'estrès hídric i recuperació en condicions naturals, establint relacions diferencials entre les fulles de ramets vegetatius i reproductors.

- *C. edulis* presenta una gran variabilitat en trets associats a la reproducció sexual tals com la producció de llavors i la germinació o longevitat d'aquestes, donant lloc a diferents dinàmiques del banc de llavors en diferents poblacions. Aquesta variabilitat està influenciada per l'ambient i la presència humana, suggerint una elevada sensibilitat a canvis en els propers anys com a conseqüència de la creixent pressió antròpica sobre l'ambient i canvi climàtic.

- La resposta hormonal de *C. edulis* en resposta a les baixes temperatures presenta plasticitat en funció de la severitat de l'estrès i el temps d'exposició, subratllant la gran capacitat d'aquesta espècie d'aclimatar-se a les baixes temperatures.

- Els individus del rang invasor de *C. edulis* presenten una major tolerància a les baixes temperatures que els individus del rang natiu. A més, el nínxol climàtic invasor de *C. edulis* ha patit una gran expansió amb relació al nínxol climàtic natiu cap a temperatures més baixes i majors condicions d'aridesa. Aquest canvi funcional contribuiria a explicar la gran expansió de l'espècie a Europa.

- La concentració de clorofil·les, el cicle de les xantofil·les i l'acumulació d'α-tocoferol són respostes comunes de *C. edulis* tant a l'estrès hídric de l'estiu com a la baixada de temperatures hivernal típica del Mediterrani.

- Els marcadors d'estrès fotooxidatiu proporcionen eines útils per a l'estudi de les invasions per tal d'avaluar efectes d'avantatges competitius davant d'espècies natives, la diferenciació entre rangs (per entendre l'adaptació al rang invasor) i la caracterització de la resposta a noves condicions ambientals.

- *C. edulis* és una espècie clonal monocàrpica a nivell de ramet però policàrpica a nivell de genet. Aquesta estratègia permet allargar la vida útil i el valor reproductor dels individus. El seu vigor vegetatiu es complementa amb una gran inversió en altres atributs com l'enorme producció de llavors.

- La senescència té un rol adaptatiu en la supervivència de *C. edulis* a tres nivells: a nivell foliar, a nivell de ramet i a nivell de genet, a través del cicle de creixement i mort que presenta aquesta espècie.

- L'èxit de la invasió de *C. edulis* rau en què el procés de la seva invasió és una retroalimentació positiva en expansió i en impactes, a través de diversos mecanismes que involucren les respostes a l'estrès, la senescència i la reproducció.

- *C. edulis* mostra variabilitat a diferents nivells que contribueixen al seu èxit invasor. La plasticitat fisiològica li permet sobreviure a nous ambients i pertorbacions. La variabilitat intraindividual entre ramets amb càrrega i sense càrrega reproductora li permet fer divisió de tasques i optimitzar els recursos de forma eficient. Finalment, la variabilitat interindividual i interpoblacional o geogràfica són reservori de possibilitats per a l'adaptació a noves condicions i a l'ambient local.

- *C. edulis* és una “invasora ideal”, però altres factors relacionats amb l’home han contribuït al seu èxit més enllà del seu transport. Entre els atributs de l’espècie que en facilitarien la invasió trobem la gran plasticitat en resposta a gradients ambientals, els atributs clonals, l’elevada inversió en reproducció sexual, els cicles de vida curts a nivell de ramet, la dinàmica en retroalimentació positiva d’expansió i impacte, i el desenvolupament d’un banc de llavors permanent. Factors humans relacionats amb l’elevada pressió de propàgul relacionada amb l’interès ornamental i l’afinitat per ambients pertorbats o amb elevada presència humana la fan en conjunt una planta invasora altament exitosa.

- Malgrat que la similitud climàtica sigui clau en algunes invasions, les prediccions de risc invasor a partir del nínxol climàtic natiu no contemplen canvis evolutius i per tant poden no ser efectives per predir zones en risc en el rang introduït. La predicció d’invasions ha de partir del nínxol introduït, incorporar aspectes mecanicistes de la resposta de la planta a l’ambient present i futur i incorporar variabilitat geogràfica.

- La restauració d’ambients degradats i l’eliminació de la pressió de propàgul lligada a les activitats humanes tals com la jardineria poden ser accions tan importants com la prevenció d’entrada d’espècies exòtiques o la seva erradicació.

- Per abordar l’erradicació de *C. edulis,* una estratègia rendible pot ser el disseny d’àrees prioritàries, prioritzant les poblacions que es troben en un estadi avançat de la retroalimentació positiva de la invasió. El test de longevitat de les llavors dissenyat per a aquesta espècie pot ser una eina efectiva per identificar poblacions prioritàries i que requeriran major vigilància a llarg termini després de l’erradicació.

Bibliografia

Bibliografia

Adams, W.W., Zarter, C.R., Ebbert, V., Demmig-Adams, B. (2004). Photoprotective strategies of overwintering evergreens. Bioscience 54(1), 41-49.

Agrawal, A.A., Johnson, M.T.J., Hastings, A.P., Maron, J.L. (2013). A field experiment demonstrating plant life-history evolution and its eco-evolutionary feedback to seed predator populations. American Naturalist 181(Suppl 1), S35-S45.

Aguirre-Gutiérrez, J., Serna-Chavez, H.M., Villalobos-Arambula, A.R., Pérez de la Rosa, J. A., Raes, N. (2015). Similar but not equivalent: Ecological niche comparison across closely-related Mexican white pines. Diversity and Distributions 21(3), 245-257.

Ahmad, P., Rasool, S., Gul, A., Sheikh, S.A., Akram, N.A., Ashraf, M., et al. (2016). Jasmonates: Multifunctional roles in stress tolerance. Frontiers in Plant Science 7, 813.

Alpert, P., Bone, E., Holzapfel, C. (2000). Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspectives in Plant Ecology 3(1), 52-66.

Apel, K., Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55, 373-399.

Aragón, C. (2014). Evolution of plant life history strategies: From Cole to contemporary invasion ecology. Ecosistemas 23(3), 6-12.

Arnao, M.B., Hernández-Ruiz, J. (2014). Melatonin: Plant growth regulator and/or biostimulator during stress? Trends in Plant Science 19(12), 789-797.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiology 141(2), 391-396.

Atwater, D.Z., Ervine, C., Barney, J.N. (2018). Climatic niche shifts are common in introduced plants. Nature Ecology and Evolution 2(1), 34-43.

Baker, H.G. (1965). Characteristics and modes of origin of weeds. A: Baker H.G., Stebbins G.L. (ed). The genetics of colonizing species; proceedings. New York: Academic Press.

Balaguer, L., Pugnaire, F.I., Martínez-Ferri, E., Armas, C., Valladares, F., Manrique, E. (2002). Ecophysiological significance of chlorophyll loss and reduced photochemical efficiency under extreme aridity in *Stipa tenacissima* L. Plant Soil 240, 343-352.

Bårdsen, B.J., Henden, J.A., Fauchald, P., Tveraa, T. Stien, A. (2011). Plastic reproductive allocation as a buffer against environmental stochasticity. Linking life history and population dynamics to climate. Oikos 120(2), 245-257.

Bartomeus, I., Bosch, J., Vilà, M. (2008). High invasive pollen transfer, yet low deposition on native stigmas in a *Carpobrotus*-invaded community. Annals of Botany 102(3), 417-424.

Bartomeus, I., Sol, D., Pino, J., Vicente, P., Font, X. (2012). Deconstructing the native-exotic richness relationship in plants. Global Ecology and Biogeography 21(5), 524-533.

Bartomeus, I., Vilà, M. (2009). Breeding system and pollen limitation of two supergeneralist alien plants invading Mediterranean shrublands. Australian Journal of Botany 57, 109-115.

Baudisch, A., Salguero-Gómez, R., Jones, O.R., Wrycza, T., Mbeau-Ache, C., Franco, M., Colchero, F. (2013). The pace and shape of senescence in angiosperms. Journal of Ecology 101(3), 596-606.

Bayón, Á., Vilà, M. (2019). Horizon scanning to identify invasion risk of ornamental plants marketed in Spain. NeoBiota 52, 47–86.

Beck, E.H., Fettig, S., Knake, C., Hartig, K. Bhattarai, T. (2007). Specific and unspecific responses of plants to cold and drought stress. Journal of Biosciences 32(3), 501-10.

Beckmann, M., Erfmeier, A., Bruelheide, H. (2009). A comparison of native and invasive populations of three clonal plant species in Germany and New Zealand. Journal of Biogeography 36(5), 865-878.

Bennett, J.A., Riibak, K., Tamme, R., Lewis, R.J., Pärtel, M. (2016). The reciprocal relationship between competition and intraspecific trait variation. Journal of Ecology 104(5), 1410-1420.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., et al. (2011). A proposed unified framework for biological invasions. Trends in Ecology and Evolution 26(7), 333-339.

Blossey, B., Notzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants. A hypothesis. Journal of Ecology 83(5), 887-889.

Bock, D.G., Kantar, M.B., Caseys, C., Matthey-Doret, R., Rieseberg, L.H. (2018). Evolution of invasiveness by genetic accommodation. Nature Ecology and Evolution 2(6), 991-999.

Bonser, S.P. (2013). High reproductive efficiency as an adaptive strategy in competitive environments. Functional Ecology 27(4), 876-885.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E., Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144(1), 1-11.

Broennimann, O., Treier, U.A., Müller-Schärer, H., Thuiller, W., Peterson, A. T., Guisan, A. (2007). Evidence of climatic niche shift during biological invasion. Ecology Letters 10(8), 701-709.

Buchholz, S., Kowarik, I. (2019). Urbanisation modulates plant-pollinator interactions in invasive vs. native plant species. Scientific Reports 9, 6375.

Calow, P. (1979). The cost of reproduction: An ecological approach. Biological Reviews 54(1), 23-40.

Campoy, J.G., Acosta, A.T.R, Affre, L., Barreiro, R., Brundu, G., Buisson, E., González, L., Lema, M., Novoa, A., Retuerto, R., Roiloa, S.R., Fagúndez., J. (2018). Monographs of invasive plants in Europe: *Carpobrotus*. Botany Letters 165(3-4), 440-475.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., et al. (2012). Biodiversity loss and its impact on humanity. Nature 486, 59-67.

Cardoso A.A., Batz T.A., McAdam, S.A.M. (2020). Xylem embolism resistance determines leaf mortality during drought in *Persea americana*. Plant Physiology 182(1), 547-554.

Catford, J.A., Jansson, R., Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity and Distributions 15(1), 22-40.

Cela, J., Tweed, J.K.S., Sivakumaran, A., Lee, M.R.F., Mur, L.A.J., Munné-Bosch, S., (2018). An altered tocopherol composition in chloroplasts reduces plant resistance to *Botrytis cinerea*. Plant Physiology and Biochemistry 127, 200-210.

Chapman, D.S., Scalone, R., Štefanić, E., Bullock, J.M. (2017). Mechanistic species distribution modeling reveals a niche shift during invasion. Ecology 98(6), 1671-1680.

Chevin, L-M., Lande, R., Mace G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biology 8(4), e1000357.

Christensen, J.H. , Hewitson, B. , Busuioc, A. , Chen, A. , Gao, X. , Held, R., et al. (2007). Regional climate projections. A: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (ed). Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, New York: Cambridge University Press.

Ciccarelli, D., Picciarelli, P., Bedini, G., Sorce, C. (2016). Mediterranean Sea cliff plants: Morphological and physiological responses to environmental conditions. Journal of Plant Ecology 9(2), 153-164.

Colautti, R.I., MacIsaac, H.J. (2004). A neutral terminology to define ‘invasive’ species. Diversity and Distributions 10(2), 135-141.

Colautti, R.I., Barrett, S.C.H. (2013). Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 18(6156), 364-366.

Cole, L.C. (1954). The population consequences of life history phenomena. The Quarterly Review of Biology 29(2), 103-137.

Conser, C., Connor, E.F. (2009). Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. Biological Invasions 11, 349-358.

Cotado, A., Munné-Bosch, S. (2020). Plasticity in the growth habit prolongs survival at no physiological cost in a monocarpic perennial at high altitudes. Annals of Botany 125(3), 413-421.

Crystal-Ornelas, R., Lockwood, J.L. (2020). The ‘known unknowns’ of invasive species impact measurement. Biological Invasions 22, 1513-1525.

Cuddington, K., Hastings, A. (2004). Invasive engineers. Ecological Modelling 178 (3-4), 335-347.

D’Antonio, C.M., Mahall, B.E. (1991). Root profiles and competition between the invasive exotic perennial, *Carpobrotus edulis* and two native shrub species in California coastal scrub. American Journal of Botany 78(7), 885-894.

Dahlgren, J.P., Roach, D.A. (2017). Demographic senescence in herbaceous plants. A: Shefferson, R.P., Jones, O.R., Salguero-Gómez, R. (ed). The evolution of senescence in the tree of life. Cambridge: Cambridge University Press.

Dall’Osto, L., Lico, C., Alric, J., Giuliano, G., Havaux, M., Bassi, R. (2006). Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection in vivo under strong light. BMC Plant Biology 6, 32.

Dawkins, R. (1976). The selfish gene. Oxford: Oxford University Press.

De la Peña, E., De Clercq, N., Bonte, D., Roiloa, S., Rodríguez-Echeverría, S., Freitas, H. (2010). Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*. Biological Invasions 12, 3637-3648.

De Witte, L.C., Scherrer, D., Stöcklin, J. (2011). Genet longevity and population age structure of the clonal pioneer species *Geum reptan*s based on demographic field data and projection matrix modelling. Preslia 83(3), 371-386.

De Witte, L.C., Stöcklin, J. (2010). Longevity of clonal plants: Why it matters and how to measure it. Annals of Botany 106(6), 859-870.

Demmig, B., Björkman, O. (1987). Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. Planta 171(2),171-184.

Demmig-Adams, B., Adams, W.W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends in Plant Science 1(1), 21-26.

Demmig-Adams, B., Adams, W.W. (1992). Photoprotection and other responses of plants to high light stress. Annual Review of Plant Physiology 43(1), 599-626.

Demmig-Adams, B., Adams, W.W. (2018). An integrative approach to photoinhibition and photoprotection of photosynthesis. Environmental and Experimental Botany 154,1-3.

Demmig-Adams, B., Stewart, J.J., Adams, W.W. (2014). Multiple feedbacks between chloroplast and whole plant in the context of plant adaptation and acclimation to the environment. Philosophical Transactions of the Royal Society B: Biological Sciences 369(1640).

Demmig-Adams, B., Stewart, J.J., Adams, WW. (2017). Environmental regulation of intrinsic photosynthetic capacity: An integrated view. Current Opinion in Plant Biology 37, 34-41.

Diugosch, K.M., Parker, I.M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17(1), 431-449.

Domínguez, M.T., Aponte, C., Pérez-Ramos, I.M., García, L.V., Villar, R., Marañón, T. (2012). Relationships between leaf morphological traits, nutrient concentrations and isotopic signatures for mediterranean woody plant species and communities. Plant and Soil, 357, 407-424.

Douce, R. Joyard, J. (1980). Plant galactolipids. A: Stumpf, P.K. (ed). Lipids: Structure and function. The biochemistry of plants. Vol. 4. Academic Press.

Early, R., Sax, D.F. (2014). Climatic niche shifts between species' native and naturalized ranges raise concern for ecological forecasts during invasions and climate change. Global Ecology and Biogeography 23(12), 1356-1365.

Earnshaw, M.J., Carver, K.A., Charlton, W.A. (1987). Leaf anatomy, water relations and crassulacean acid metabolism in the chlorenchyma and colourless internal water-storage tissue of *Carpobrotus edulis* and *Senecio ?mandraliscae*. Planta 170,421-32.

Elton, C.S. (1958). The ecology of invasions by animals and plants. New York: Springer.

Enders, M., Havemann, F., Jeschke, J. M. (2019). A citation-based map of concepts in invasion biology. NeoBiota 42, 23-42.

Enders, M., Havemann, F., Ruland, F., Bernard-Verdier, M., Catford, J.A., Gómez-Aparicio, L., et al. (2020). A conceptual map of invasion biology: Integrating hypotheses into a consensus network. Global Ecology and Biogeography 29(6), 978-991.

Enders, M., Hütt, M.-T., Jeschke, J.M. (2018). Drawing a map of invasion biology based on a network of hypotheses. Ecosphere 9, e02146.

Eriksson, O. (1992). Evolution of seed dispersal and recruitment in clonal plants. Oikos 63(3), 439-448.

Esler, K.J., Jacobsen, A.L., Pratt, R.B. (2018). The biology of Mediterranean-type ecosystems. Oxford University Press.

Esteban, R., Barrutia, O., Artetxe, U., Fernández-Marín, B., Hernández, A., García-Plazaola, J.I. (2015). Internal and external factors affecting photosynthetic pigment composition in plants: A meta-analytical approach. New Phytologist 206(1), 268-280.

Esteban, R., Olano, J.M., Castresana, J., Fernández-Marín, B., Hernández, A., Becerril, J.M., García-Plazaola, J.I. (2009). Distribution and evolutionary trends of photoprotective isoprenoids (xanthophylls and tocopherols) within the plant kingdom. Physiologia Plantarum 135(4), 379-389.

Evans, T.G., Diamond, S.E., Kelly, M.W. (2015). Mechanistic species distribution modelling as a link between physiology and conservation. Conservation Physiology 3(1), cov056.

Falk, J., Munné-Bosch, S. (2010). Tocochromanol functions in plants: antioxidation and beyond. Journal of Experimental Botany 61(6), 1549-1566.

Fernández-Marín, B., Hernández, A., Garcia-Plazaola, J.I., Esteban, R., Míguez, F., Artetxe, U., Gómez-Sagasti, M.T. (2017). Photoprotective strategies of Mediterranean plants in relation to morphological traits and natural environmental pressure: A meta-analytical approach. Frontiers in Plant Science 8, 1051.

Fernández-Marín, B., Nadal, M., Gago, J., Fernie, A.R, López-Pozo, M., Artetxe, U., García-Plazaola, J.I., Verhoeven, A. (2020). Born to revive: molecular and physiological mechanisms of double tolerance in a paleotropical and resurrection plant. New Phytologist 226(3)741-759.

Fischer, A.G. (1960). Latitudinal variations in organic diversity. Evolution 14, 64.

Fraga, P., Estaún, I., Olives, J., Da Cunha, G., Alarcón, A., Cots, R., Juaneda, J., Riudavets, X. (2005). Eradication of *Carpobrotus* (L.) N. E. Br. In Minorca. A: Invasive plants in Mediterranean type regions of the world. Proceedings of the International Workshop, editat per S. Brunel, 203-208. Strasbourg: Council of Europe Publishing.

Funk, J.L. (2013). The physiology of invasive plants in low-resource environments. Conservation Physiology 1(1), cot026.

Funk, J.L. (2008). Differences in plasticity between invasive and native plants from a low resource environment. Journal of Ecology 96(6), 1162-1173.

Funk, J.L., Parker, I.M., Matzek, V., Flory, S.L., Aschehoug, E.T., D'Antonio, C.M. et al. (2020). Keys to enhancing the value of invasion ecology research for management. Biological Invasions (2020).

Gadgil, M., Bossert, W.H., (1970). Life historical consequences of natural selection. The American Naturalist 104(935), 1-24.

Gallagher, R.V., Beaumont, L.J., Hughes, L., Leishman, M.R. (2010). Evidence for climatic niche and biome shifts between native and novel ranges in plant species introduced to Australia. Journal of Ecology 98(4), 790-799.

Gao, H., Zhang, Z.K., Chai, H.K., Cheng N., Yang Y., Wang D.N. et al. (2016). Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. Postharvest Biology and Technology 118, 103-110.

Gardner, S.N., Mangel, M. (1999). Modeling investments in seeds, clonal offspring, and translocation in a clonal plant. Ecology 80(4), 1202-1220.

Giejsztowt, J., Classen, A.T., Deslippe, J.R. (2020). Climate change and invasion may synergistically affect native plant reproduction. Ecology 101(1), e02913.

Giorgi, F. (2006). Climate change hot-spots. Geophysical Reserch Letters 33(8), L08707.

Gioria, M., Pyšek, P. (2016). The legacy of plant invasions: Changes in the soil seed bank of invaded plant communities. Bioscience 66(1), 40-53.

Gioria, M., Pyšek, P., Moravcová, L. (2012). Soil seed banks in plant invasions: Promoting species invasiveness and long-term impact on plant community dynamics. Preslia 84, 327-350.

Goh, C., Ko, S., Koh, S., Kim, Y.J., Bae, H.J. (2012). Photosynthesis and environments: Photoinhibition and repair mechanisms in plants. Journal of Plant Biology 55, 93-101.

Grant, O.M., Tronina, Ł., García-Plazaola, J.I., Esteban, R., Pereira, J.S., Manuela Chaves, M. (2015). Resilience of a semi-deciduous shrub, *Cistus salvifolius*, to severe summer drought and heat stress. Functional Plant Biology 42, 219-228.

Grinnell, J. (1917). The niche-relationships of the California thrasher. The Auk 34(4), 427-433.

Groves, R.H., Panetta, F.D. (2002). Some princples for weed eradication programs. Pages 307-310. A: Spaford Jacob H., Dodd J., Moore J.H. (eds). Thirteenth Australian Weeds Conference. Plant protection society of western Australia. Victoria Park, W.A., Australia.

Guirado, M. et al. (2017). Protocols de gestió de la flora exòtica invasora. 6. Control de l'ungla de gat. Diputació de Girona / Àrea de Territori i Sostenibilitat / Medi Ambient.

Guisan, A., Petitpierre, B., Broennimann, O., Daehler, C., Kueffer, C. (2014). Unifying niche shift studies: Insights from biological invasions. Trends in Ecology and Evolution 29(5), 260-269.

Gulías, J., Flexas, J., Abadia, A., Medrano, H. (2002). Photosynthetic responses to water deficit in six Mediterranean sclerophyll species: Possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. Tree Physiology 22(10), 687-697.

Haeuser, E., Dawson, W., Thuiller, W., Dullinger, S., Block, S., Bossdorf, O., et al. (2018). European ornamental garden flora as an invasion debt under climate change. Journal of Applied Ecology 55(5), 2386- 2395.

Hahn, M.A., Müller-Schärer, H. (2013). Cytotype differences modulate eco-geographical differentiation in the widespread plant *Centaurea stoebe*. Ecology 94, 1005-1014.

Harman, D. (1992). Free radical theory of aging. Mutation Research 275(3-6), 257-266.

Harper, J.L. (1977). Population biology of plants. New York: Academic Press.

Hartnett, D.C. (1987). Effects of fire on clonal growth and dynamics of *Pityopsis graminifolia* (Asteraceae). American Journal of Botany, 74(11), 1737-1743.

Hasegawa, S., Takeda, H. (2001). Functional specialization of current shoots as a reproductive strategy in Japanese alder (*Alnus shirsuta var. sibirica*). Canadian Journal of Botany 79(1), 38-48.

Hayden, S.H., White, P. (2001). Horticulture as a pathway of invasive plant introductions in the United States: Most invasive plants have been introduced for horticultural use by nurseries, botanical gardens, and individuals. Bioscience 51(2), 103-113.

Hayflick, L. (1965). The limited in vitro lifetime of human diploid cell strains. Experimental Cell Research 37(3), 614-636.

Heger, T., Pahl, A.T., Botta-Dukát, Z., Gherardi, F., Hoppe, C., Hoste, I. et al. (2013). Conceptual frameworks and methods for advancing invasion ecology. Ambio 42, 527-540.

Hernández, I., Alegre, L., Munné-Bosch, S., (2004). Drought-induced changes in flavonoids and other low molecular weight antioxidants in *Cistus clusii* grown under Mediterranean field conditions. Tree Physiology 24(11), 1303-1311.

Herrera, C.M. (2017). The ecology of subindividual variability in plants: Patterns, processes, and prospects. Web Ecology 17(2), 51-64.

Herrera, C.M., Medrano, M., Bazaga, P. (2015). Continuous within-plant variation as a source of intraspecific functional diversity: Patterns, magnitude, and genetic correlates of leaf variability in *Helleborus foetidus* (Ranunculaceae). American Journal of Botany 102(2), 225-232.

Higgins, S.I., Richardson, D.M. (2014). Invasive plants have broader physiological niches. PNAS 111(29), 10610-10614.

Hobbs, R.J. (2000). Land-use changes and invasions. A: Mooney, H.A., Hobbs, R.J. (ed) Invasive species in a changing world. Washington DC: Island Press.

Hobbs, R.J., Huenneke, L.F. (1992). Disturbance, diversity, and invasion: Implications for conservation. Conservation Biology 6(3), 324-337.

Huang, Q.Q., Pan, X.Y., Fan, Z.W., Peng, S.L. (2015). Stress relief may promote the evolution of greater phenotypic plasticity in exotic invasive species: A hypothesis. Ecology and Evolution 5(6), 1169-1177.

Hueso Alcaide, M.C. (2017). Evolución del glifosato en suelo y plantas de la devesa de L'Albufera de Valencia tras su aplicación para el control de *Carpobrotus edulis*. Parc Natural de L'Albufera.

Hulme, P.E. (2006). Beyond control: Wider implications for the management of biological invasions. Journal of Applied Ecology 43(5), 835-847.

Hulme, P.E., Brundu, G., Carboni, M., Dehnen-Schmutz, K., Dullinger, S., Early R., et al. (2018). Integrating invasive species policies across ornamental horticulture supply chains to prevent plant invasions. Journal of Applied Ecology 55(1), 92- 98.

Humphreys, A.M., Govaerts, R., Ficinski, S.Z., Nic Lughadha, E., Vorontsova, M.S. (2019). Global dataset shows geography and life form predict modern plant extinction and rediscovery. Nature Ecology and Evolution 3, 1043-1047.

Hutchinson, G.E. (1957). Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22, 415-427.

Inderjit, Callaway, R.M., Vivanco, J.M. (2006). Can plant biochemistry contribute to understanding of invasion ecology? Trends in Plant Science 11, 574-580.

Jakobsson, A., Padrón, B., Traveset, A. (2008). Pollen transfer from invasive *Carpobrotus* spp. to natives - A study of pollinator behaviour and reproduction success. Biological Conservation 141(1), 136-145.

Jelbert, K., Stott, I., Mcdonald, R.A., Hodgson, D. (2015). Invasiveness of plants is predicted by size and fecundity in the native range. Ecology and Evolution 5(10), 1933-1943.

Jeschke, J.M. (2014). General hypotheses in invasion ecology. Diversity and Distributions 20(11), 1229-1234.

Jeschke, J.M., Heger, T. (ed) (2018). Invasion biology: Hypotheses and evidence. CABI, Wallingford, UK.

Jibran, R., Hunter, D.A., Dijkwel, P.P. (2013). Hormonal regulation of leaf senescence through integration of developmental and stress signals. Plant Molecular Biology 82(6), 547-561.

Johnson, M.T.J. (2007). Genotype-by-environment interactions leads to variable selection on life-history strategy in common evening primrose (*Oenothera biennis*). Journal of Evolutionary Biology 20(1), 190-200.

Jönsson, K.I. (2000). Life history consequences of fixed costs of reproduction. Écoscience 7(4), 423-427.

Joshi, S., Tielbörger, K. (2012). Response to enemies in the invasive plant *Lythrum salicaria* is genetically determined. Annals of Botany 110(7), 1403-1410.

Jucker, T., Carboni, M., Acosta, A.T.R. (2013). Going beyond taxonomic diversity: Deconstructing biodiversity patterns reveals the true cost of iceplant invasion. Diversity and Distributions 19(12), 1566-1577.

Juvany, M., Müller, M., Munné-Bosch, S. (2013). Photo-oxidative stress in emerging and senescing leaves: A mirror image? Journal of Experimental Botany 64(11), 3087-3098.

Kawecki, T.J., Ebert, D. (2004). Conceptual issues in local adaptation. Ecology Letters 7(12), 1225-1241.

Kazan, K. (2015). Diverse roles of jasmonates and ethylene in abiotic stress tolerance. Trends in Plant Science 20(4), 219-229.

Kearney, M., Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. Ecology Letters 12(4), 334-350.

Keesing, F., Belden, L.K., Daszak, P., Dobson, A., Harvell, C.D., Holt, R.D., et al. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468, 647-652.

Kim, T.H., Böhmer, M., Hu, H., Nishimura, N., Schroeder, JI. (2010). Guard cell signal transduction network: Advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. Annual Review in Plant Biology 61, 561-591.

Klausmeyer, K.R., Shaw, M.R. (2009). Climate change, habitat loss, protected areas and the climate adaptation potential of species in Mediterranean ecosystems worldwide. PLoS One 4(7), e6392.

Kleine, S., Weissinger, L., Müller, C. (2017). Impact of drought on plant populations of native and invasive origins. Oecologia 183(1), 9-20.

Klimešová, J., Ottaviani, G., Martínková, J. (2020). Switching from monocarpic to polycarpic perennial life histories in a cold climate: A commentary on “Physiological costs of clonal growth.” Annals of Botany 125(3), 4-5.

Koca, I., Koca, A.F. (2007). Poisoning by mad honey: A brief review. Food and Chemical Toxicology 45(8), 1315-1318.

Köppen, V.W. (1923). Die Klimate der Erde. Grundriss der Klimakunde. Quarterly Journal of the Royal Meteorological Society 50, 161-162.

Krieger-Liszkay, A. (2005). Singlet oxygen production in photosynthesis. Joural of Experimental Botany 56(411), 337-346.

Kueffer, C. (2017). Plant invasions in the Anthropocene. Science 358(6364), 724-725.

Kumar Rai, P., Singh, J.S. (2020). Invasive alien plant species: Their impact on environment, ecosystem services and human health. Ecological Indicators 111, 106020.

Kumar, S., Saxena, S., Rai, A., Radhakrishna, A., Kaushal, P. (2019). Ecological, genetic, and reproductive features of *Cenchrus* species indicate evolutionary superiority of apomixis under environmental stresses. Ecological Indicators 105, 126-136.

Kyparissis, A., Petropoulou, Y., Manetas, Y. (1995). Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa L., Labiatae*) under Mediterranean field conditions: Avoidance of photoinhibitory damage through decreased chlorophyll contents. Journal of Experimental Botany 46(12), 1825-1831.

Lambers, H., Oliveira, R.S. (2019). Plant physiological ecology. Third edition. Springer International Publishing.

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. Journal of Evolutionary Biology 22(7), 1435-1446.

Lavergne, S., Thompson, J.D., Garnier, E., Debussche, M. (2004). The biology of endemic and widespread plants: A comparative study of trait variation in 20 congeneric pairs. Oikos 107(3), 505-51.

Lawrence, E.R., Fraser, D.J. (2020). Latitudinal biodiversity gradients at three levels: Linking species richness, population richness and genetic diversity. Global Ecology and Biogeography 29(5), 770-788.

Lazzaro, L., Giuliani, C., Fabiani, A., Agnelli, A. E., Pastorelli, R., Lagomarsino, A., et al. (2014). Soil and plant changing after invasion: The case of *Acacia dealbata* in a Mediterranean ecosystem. Science of the Total Environment 1, 497-498.

Lazzaro, L., Tondini, E., Lombardi, L., Giunti, M. (2020). The eradication of *Carpobrotus* spp. in the sand-dune ecosystem at Sterpaia (Italy, Tuscany): Indications from a successful experience. Biologia 75, 199-208.

Lear, L., Hesse, E., Shea, K., Buckling, A. (2020). Disentangling the mechanisms underpinning disturbance mediated invasion. Proceedings of the Royal Society B 287(1919).

Lechuga-Lago, Y., Novoa, A., Le Roux, J.J., Gonzalez, L. (2017). Understanding the influence of urbanization on invasibility: *Carpobrotus edulis* as an exemplar. Biological Invasions 19, 3601-3611.

Leimu, R., Fischer, M. (2008). A meta-analysis of local adaptation in plants. PLoS ONE 3, 12 e4010.

Levine, J.M., D'Antonio, C.M. (1999). Elton revisited: A review of evidence linking diversity and invasibility. Oikos 87(1), 15-26.

Levins, R. (1968). Evolution in changing environments. Princeton University Press, Princeton.

Levins, R., MacArthur, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. The American Naturalist 101(921), 377-385.

Levitt, J. (1980). Responses of plants to environmental stresses. New York: Academic Press.

Lockwood, J.L., Cassey, P., Blackburn, T. (2005). The role of propagule pressure in explaining species invasions. Trends in Ecology and Evolution 20(5), 223-228.

Lodge, D.M. (1993). Biological invasions: Lessons for ecology. Trends in Ecology and Evolution 8(4), 133-137.

Ludlow, M.M. (1989). Strategies of response to water stress. A: Structural and funcional responses to evironmental stresses: water shortage. Berlin: SPB Academic Publishers, pp. 269-281.

Lynch, M., Bürger, R., Butcher, D., Gabriel, W. (1993). The mutational meltdown in asexual populations. Journal of Heredity 84(5), 339-344.

MacArthur, R. (1970). Species packing and competitive equilibrium for many species. Theoretical Population Biology 1(1), 1-11.

Margalef, R. (1957). La teoría de la información en ecología. Memorias de la Real Academia de Ciencias y Artes de Barcelona 35.

Matesanz, S., Valladares, F. (2014). Ecological and evolutionary responses of Mediterranean plants to global change. Environmental and Experimental Botany 103, 53-67.

Matzek, V., Pujalet, M., Cresci, S. (2015). What managers want from invasive species research versus what they get. Conservation Letters 8(1), 33-40.

Mazza, G., Tricarico, E., Genovesi, P., Gherardi, F. (2014). Biological invaders are threats to human health: An overview. Ethology, Ecology and Evolution 26(2-3), 112-129.

Medawar, P.B. (1952). An unsolved problem of biology. London: H.K. Lewis.

Metz, J., Liancourt, P., Kigel, J., Harel, D., Sternberg, M. and Tielbörger, K. (2010). Plant survival in relation to seed size along environmental gradients: a long-term study from semi-arid and Mediterranean annual plant communities. Journal of Ecology 98(3), 697-704.

Míguez, F., Fernández-Marín, B., Becerril, J.M., García-Plazaola, J.I. (2015). Activation of photoprotective winter photoinhibition in plants from diferent environments: A literature compilation and meta-analysis. Physiologia Plantarum 155(4), 414-423.

Milanović, M., Knapp, S., Pyšek, P., Kühn, I., (2020). Linking traits of invasive plants with ecosystem services and disservices. Ecosystem Services 42, 101072.

Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos, J.N., et al. (2006). Biotic interactions and plant invasions. Ecology Letters 9(6), 726-740.

Mitrakos, K. (1982). Winter low temperatures in Mediterranean-type ecosystems. Bioclimatic aspects, plant reactions to winter cold. Ecologia Mediterranea 8, 95-102.

Mittermeier, R.A., Gil, P.G., Hoffman, M.T., Pilgrim, J.D., Brooks, T.M., Mittermeier, C.G., et al. (2004). Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. Cemex.

Molinari, N., D'Antonio, C., Thomson, G. (2007). *Carpobrotus* as a case study of the complexities of species impacts. Chapter 7. A: Cuddington K., Byers J.E., Wilson W.G., Hastings A. (ed). Ecosystem engineers: Plants to protists. Theoretical ecology series. Vol. 4. London: Academic Press.

Moragues, E., Travesset, A. (2005). Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. Biological Conservation 122(4), 611-619.

Moravcová, L., Pyšek, P., Jarošík, V., Pergl, J. (2015). Getting the right traits: Reproductive and dispersal characteristics predict the invasiveness of herbaceous plant species. PLoS ONE 10(4), e0123634.

Morosinotto, T., Bassi, R. (2012). Assembly of light harvesting pigment-protein complexes in photosynthetic eukaryotes. A: Eaton-Rye, J.J., Tripathy, B.C., Sharkey, T.D. (eds). Photosynthesis. Plastid biology, energy conversion and carbon assimilation. Dordrecht: Springer.

Munné-Bosch, S. (2018). Limits to tree growth and longevity. Trends in Plant Science 23(11), 985-993.

Munné-Bosch, S., Alegre, L. (2000). Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210(6), 925-931.

Munné-Bosch, S., Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. Critical Reviews in Plant Sciences 21(1), 31-57.

Munné-Bosch, S., Alegre, L. (2004). Die and let live: Leaf senescence contributes to plant survival under drought stress. Functional Plant Biology 31(3), 203-216.

Munné-Bosch, S., Jubany-Marí, T., Alegre, L. (2001). Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. Plant Cell and Environment 24(12), 1319-1327.

Munné-Bosch, S., Peñuelas, J. (2003). Photo- and antioxidative protection during summer leaf senescence in *Pistacia lentiscus* L. grown under Mediterranean field conditions. Annals of Botany 92(3), 385-391.

Muoghalu, J.I. (2008). Growth, reproduction and resource allocation of *Tithonia diversifolia* and *Tithonia rotundifolia*. Weed Research 48(2), 157-162.

Myers, N., Mittermeler, R.A., Mittermeler, C.G., da Fonseca, G.A.B., Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature 403, 853-858.

Najberek, K., Pusz, W., Solarz, W., Olejniczak, P. (2018). The seeds of success: release from fungal attack on seeds may influence the invasiveness of alien *Impatiens*. Plant Ecology 219(10), 1197-1207.

Nakayama, H., Sinha, N.R., Kimura, S. (2017). How do plants and phytohormones accomplish heterophylly, leaf phenotypic plasticity, in response to environmental cues. Frontiers in Plant Science 8, 1717.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., et al. (2010). Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15(12), 684-692.

Nisar, N., Li, L., Lu, S., Khin, N.C., Pogson, B.J. (2015) Carotenoid metabolism in plants. Molecular Plant 8(1), 68-82.

Northfield, T.D., Laurance, S.G.W., Mayfield, M.M., Paini, D.R., Snyder, W.E., Stouffer, D.B., et al. (2018). Native turncoats and indirect facilitation of species invasions. Proceedings of the Royal Society B: Biological Sciences 285(1871).

Novoa, A., González, L. (2014). Impacts of *Carpobrotus edulis* (L.) NE Br. on the germination, establishment and survival of native plants: A clue for assessing its competitive strength. PLoS ONE 9 (9), e107557.

Novoa, A., Rodríguez, R., Richardson, D.M., González, L. (2014). Soil quality: A key factor in understanding plant invasion? The case of *Carpobrotus edulis* (L.) N.E.Br. Biological Invasions 16(2), 429-443.

Nyasembe, V.O., Cheseto, X., Kaplan, F., Foster, W.A., Teal, P.E.A., Tumlinson, J.H., et al. (2015). The invasive American weed *Parthenium hysterophorus* can negatively impact malaria control in Africa. PLoS ONE 10(9), e0137836.

Obeso, J.R. (2002). The costs of reproduction in plants. New Phytologist 155(3), 321-348.

Oliveira, G., Peñuelas, J. (2004). Effects of winter cold stress on photosynthesis and photochemical efficiency of PSII of the Mediterranean *Cistus albidus* L. and *Quercus ilex* L. Plant Ecology 175(2), 179-191.

Otero, I., Farrell, K.N., Pueyo, S., Kallis, G., Kehoe, L., Haberl, H., et al. (2020). Biodiversity policy beyond economic growth. Conservation Letters, e12713.

Otto, S.P. (2018). Adaptation, speciation and extinction in the Anthropocene. Proceedings of the Royal Society B: Biological Sciences 285(1891).

Pan, X.Y., Geng, Y.P., Sosa, A., Zhang, W.J., Li, B., Chen, J.K. (2007). Invasive *Alternanthera philoxeroides*: Biology, ecology and management. Acta Phytotaxonomica Sinica 45(06), 884-900.

Pearman, P.B., Guisan, A., Broennimann, O., Randin, C.F. (2008). Niche dynamics in space and time. Trends in Ecology and Evolution 23(3), 149-158.

Petitpierre, B., Kueffer, C., Broennimann, O., Randin, C., Daehler, C., Guisan, A. (2012). Climatic niche shifts are rare among terrestrial plant invaders. Science 335(6074), 1344-1348.

Phillips, B.L. (2015). Evolutionary processes make invasion speed difficult to predict. Biological Invasions 17(7), 1949-1960.

Pimentel, D., Zuniga, R., Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52(3), 273-288.

Pintó-Marijuan, M., Munné-Bosch, S. (2013). Ecophysiology of invasive plants: Osmotic adjustment and antioxidants. Trends in Plant Science 18(12), 660-666.

Pluess, A.R. Stöcklin, J. (2005). The importance of population origin and environment on clonal and sexual reproduction in the alpine plant *Geum reptans*. Functional Ecology 19(2), 228-237.

Pospíšil, P., Yamamoto, Y. (2017). Damage to photosystem II by lipid peroxidation products. Biochimica et Biophysica Acta 1861(2), 457-466.

Preston, C.D., Sell. P.D. (1988). The aizoaceae naturalized in the British Isles. Watsonia 17(3), 217-245.

Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U., et al. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: The interaction of impact measures, invading species' traits and environment. Global Change Biology 18(5), 1725-1737.

Pyšek, P., Jarošík, V., Pergl, J., Randall, R., Chytrý, M., Kühn, I., et al. (2009). The global invasion success of central european plants is related to distribution characteristics in their native range and species traits. Diversity and Distributions 15(5), 891-903.

Pyšek, P., Manceur, A.M., Alba, C., McGregor, K.F., Pergl, J., Štajerová, K., et al. (2015). Naturalization of central european plants in North America: Species traits, habitats, propagule pressure, residence time. Ecology 96(3), 762-774.

Pyšek, P., Richardson, D.M. (2007). Traits associated with invasiveness in alien plants: Where do we stand? A: Biological Invasions. Berlin Heidelberg: Springer.

Quirino, B.F., Noh, Y., Himelblau, E. Amasino, R.M. (2000). Molecular aspects of leaf senescence. Trends in Plant Science 5(7), 278-282.

Raj, S. Bräutigam, K., Hamanishi, E.T., Wilkins, O., Thomas, B.R., Schroeder, W. et al. (2011) Clone history shapes Populus drought responses. PNAS 108(30), 12521-12526.

Ramel, F., Birtic, S., Cuiné, S., Triantaphylidès, C., Ravanat, J.-L., Havaux, M. (2012). Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiology 158(3), 1267-1278.

Reinhart, K.O., Callaway, R.M. (2004). Soil biota facilitate exotic Acer invasions in Europe and North America. Ecological Applications 14(6), 1737-1745.

Rejmánek, M. (2014). Biological invasions and ecological hypotheses. Trends in Ecology and Evolution 29(8), 435.

Richards, E.J. (2006). Inherited epigenetic variation: Revisiting soft inheritance. Nature Reviews 7(5), 395-402.

Richardson, D.M., Pyšek, P. (2006). Plant invasions: Merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography: Earth and Environment 30(3), 409-431.

Richardson, D.M., Pyšek, P. (2008). Fifty years of invasion ecology. The legacy of Charles Elton. Diversity and Distributions 14(2), 161-168.

Richardson, D.M., Pyšek, P., Rejmánek M., Barbour, M.G., Panetta, F.D., West, C.J. (2000). Naturalization and invasion of alien plants: Concepts and definition. Diversity and Distributions 3(6), 93-107.

Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S., Blumwald, E. (2007). Delayed leaf senescence induces extreme drought tolerance in a flowering plant. PNAS 104(49), 19631-19636.

Roach, D.A., Smith, E.F. (2020). Life-history trade-offs and senescence in plants. Functional Ecology 34(1), 17- 25.

Rodríguez-Caballero, G., Caravaca, F., Díaz, G., Torres, P., Roldán, A. (2020). The invader *Carpobrotus edulis* promotes a specific rhizosphere microbiome across globally distributed coastal ecosystems. Science of the Total Environment 719, 137347.

Roiloa, S.R. (2019). Clonal traits and plant invasiveness: The case of *Carpobrotus* N.E.Br. (Aizoaceae). Perspectives in Plant Ecology, Evolution and Systematics 40, 125479.

Roiloa, S.R., Retuerto, R., Campoy, J.G., Novoa, A., Barreiro, R. (2016). Division of labor brings greater benefits to clones of *Carpobrotus edulis* in the non-native range: Evidence for rapid adaptive evolution. Frontiers in Plant Science 7, 349.

Roiloa, S.R., Rodríguez-Echeverría, S., Freitas, H., Retuerto, R. (2013). Developmentally-programmed division of labour in the clonal invader *Carpobrotus edulis*. Biological Invasions 15, 1895-1905.

Rose, M.R. (1991). Evolutionary biology of aging. New York: Oxford University Press.

Ruffino, L., Krebs E., Passetti A., Aboucaya A., Affre L., Fourcy D., Lorvelec O., et al. (2015). Eradications as scientific experiments: Progress in simultaneous eradications of two major invasive taxa from a Mediterranean island. Pest Management Science 71(2), 189-198.

Sánchez-Vilas, J., Retuerto, R. (2011). Reproduction reduces photosynthetic capacity in females of the subdioecious Honckenya peploides. Acta Oecologica 37(2), 155-163.

Sanz Elorza, M., Dana Sánchez, E. D., Sobrino Vesperinas, E. (2004). Plantas alóctonas invasoras en España. Madrid: Dirección General para la Biodiversidad.

Schmid, B. (1990). Some ecological and evolutionary consequences of modular organization and clonal growth in plants. Evolutionary Trends in Plants 4(1), 25-34.

Scott, I.M., Clarke, S.M., Wood, J.E., Mur, L.A.J. (2004). Salicylate accumulation inhibits growth at chilling temperature in Arabidopsis. Plant Physiology 135(2), 1040-1049.

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M. et al. (2018). Global rise in emerging alien species results from increased accessibility of new source pools. PNAS 115(10), E2264-E2273.

Shackleton, R.T., Witt, A.B.R., Piroris, F.M., van Wilgen, B.W. (2017). Distribution and socio-ecological impacts of the invasive alien cactus *Opuntia stricta* in eastern Africa. Biological Invasions 19, 2427-2441.

Shang, L., Qiu, S., Huang, J., Li, B. (2015). Invasion of *Spartina alterniflora* in China is greatly facilitated by increased growth and clonality: A comparative study of native and introduced populations. Biological Invasions 17, 1327-1339.

Sheffield, J., Wood, E.F. (2008). Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 Simulations. Climate Dynamics 31(1), 79-105.

Sher, A.A., Hyatt, L.A. (1999). The disturbed resource-flux invasion matrix: A new framework for patterns of plant invasion. Biological Invasions 1, 107-114.

Shi, J., Macel, M., Tielbörger, K., Verhoeven, K.J.F. (2018). Effects of admixture in native and invasive populations of *Lythrum salicaria*. Biological Invasions 20, 2381-2393.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., et al. (2013). Impacts of biological invasions: What's what and the way forward. Trends in Ecology and Evolution 28(1), 58-66.

Simberloff, D., von Holle, B. (1999). Positive interactions of nonindigenous species: Invasional meltdown? Biological Invasions 1, 21-32.

Simcha, L.Y. (2017). How monocarpic is Agave? Flora 230, 12-13.

Sinclair, J.S., Lockwood, J.L., Hasnain, S., Cassey, P., Arnott, S.E. (2020). A framework for predicting which non-native individuals and species will enter, survive, and exit human-mediated transport. Biological Invasions 22, 217-231.

Smith, A.L., Hodkinson, T.R., Villellas, J., Catford, J.A., Csergő, A.M., Blomberg, S.P. et al. (2020). Global gene flow releases invasive plants from environmental constraints on genetic diversity. PNAS 117(8), 4218-4227.

Smith, L.M., Reynolds H.L. (2015). *Euonymus fortunei* dominance over native species may be facilitated by plant-soil feedbak. Plant Ecology 216, 1401-1406.

Soliman, M.H., Alayaf, A.A.M., El Kelish, A.A.E., Abu-Elsaoud, A.M. (2018). Acetylsalicylic acid enhance tolerance of *Phaseolus vulgaris* L. to chilling stress, improving photosynthesis, antioxidants and expression of cold stress responsive genes. Botanical Studies 59(1), 6.

Song, Y., Yu, F.-H., Keser, L.H., Dawson, W., Fisher, M, Dong, M., et al. (2013). United we stand, divided we fall: A meta-analysis of experiments on clonal Integration and its relationship to invasiveness. Oecologia 171(2), 317-327.

Souza-Alonso, P., Guisande-Collazo, A., Lechuga-Lago, Y., González, L. (2018). The necessity of surveillance. Medium-term viability of *Carpobrotus edulis* propagules after plant fragmentation. Plant Biosystems 153(5),1-4.

Souza-Alonso, P., Lechuga-Lago, Y., Guisande-Collazo, A., Pereiro Rodríguez, D., Rosón Porto, G., González Rodríguez, L. (2020). Drifting away. Seawater survival and stochastic transport of the invasive *Carpobrotus edulis.* Science of the Total Environment 712, 135518.

Stearns, S.C. (1992). The evolution of life histories. Oxford: Oxford University Press.

Stöcklin, J., Winkler, E. (2004). Optimum reproduction and dispersal strategies of a clonal plant in a metapopulation: A simulation study with *Hieracium pilosella*. Evolutionary Ecology 18, 563-584.

Stone, C.M., Witt, A.B.R., Walsh, G.C., Foster, W.A., Murphy, S.T. (2018). Would the control of invasive alien plants reduce malaria transmission? A review. Parasites and Vectors 11(76), 1-18.

Suehs, C.M., Affre, L., Médail F. (2004). Invasion dynamics of two alien *Carpobrotus* (Aizoaceae) taxa on a Mediterranean Island: II. Reproductive Strategies. Heredity 92(6), 550-556.

Sun, Y., Roderick, G.K. (2019). Rapid evolution of invasive traits facilitates the invasion of common ragweed, *Ambrosia artemisiifolia*. Journal of Ecology 107(6), 2673-2687.

Takahashi, S., Badger, M.R. (2011). Photoprotection in plants: A new light on photosystem II damage. Trends in Plant Science 16(1), 53-60.

Taylor, K.T., Maxwell, B.D., Pauchard, A., Nuñez, M.A., Peltzer, D.A., Terwei, A., et al. (2016). Drivers of plant invasion vary globally: Evidence from pine invasions within six ecoregions. Global Ecology and Biogeography 25(1), 96-106.

Theoharides, K.A., Dukes, J.S. (2007). Plant invasion across space and time: Factors affecting nonindigenous species success during four stages of invasion. New Phytologist 176(2), 256-273.

Thomas, H. (2013). Senescence, ageing and death of the whole plant. New Phytologist 197(3), 696-711.

Thomas, H., Thomas, H.M., Ougham, H. (2000). Annuality, perenniality and cell death. Journal of Experimental Botany 51(352), 1781-1788.

Thuiller, W., Richardson, D.M., Pyšek, P., Midgley G.F., Hugues, G.O., Rouget, M. (2005). Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. Global Change Biology 11(12), 2234-2250.

Tilman, D., Reich, P.B., Knops, J.M.H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441, 629-632.

Traveset, A., Moragues, E., Valladares, F. (2008). Spreading of the Invasive *Carpobrotus aff. Acinaciformis* in Mediterranean ecosystems: The advantage of performing in different light environments. Applied Vegetation Science 11(1), 45-54.

Triantaphylidès, C., Havaux, M. (2009). Singlet oxygen in plant, production, detoxification and signaling. Trends in Plant Science 14(4), 219-228.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., et al. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecology Letters 17(11), 1351-1364.

Valladares, F., Sanchez-Gomez, D. Zavala, M.A. (2006). Quantitative estimation of phenotypic plasticity: Bridging the gap between the evolutionary concept and its ecological applications. Journal of Ecology 94(6), 1103-1116.

van Baalen, J., Prins, E.G.M. (1983). Growth and reproduction of *Digitalis purpurea* in different stages of succession. Oecologia 58(1), 84-91.

Van Drunen, W.E. Dorken, M.E. (2012). Trade-offs between clonal and sexual reproduction in *Sagittaria latifolia* (Alismataceae) scale up to affect the fitness of entire clones. New Phytologist, 196(2), 606-616.

van Kleunen, M., Essl, F., Pergl, J., Brundu, G., Carboni, M., Dullinger, S., et al. (2018). The changing role of ornamental horticulture in alien plant invasions. Biological Reviews 93(3), 1421-1437.

van Kleunen, M., Weber, E., Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters 13(2), 235-245.

Vaz, A.S., Kueffer, C., Kull, C. A., Richardson, D.M., Schindler, S., Muñoz-Pajares, A.J., et al. (2017). The progress of interdisciplinarity in invasion science. Ambio 46(4), 428-442.

Verhoeven, A., García-Plazaola, J.I., Fernández-Maríın, B. (2018) Shared mechanisms of photoprotection in photosynthetic organisms tolerant to desiccation or to low temperature. Environmental and Experimental Botany 154: 66–79

Verhoeven, K.J.F., Preite, V. (2013). Epigenetic variation in asexually reproducing organisms. Evolution 68(3), 644-655.

Verhoeven, K.J.F., Biere, A., Harvey, J.A., van der Putten, W. H. (2009). Plant invaders and their novel natural enemies: Who is naïve? Ecology Letters 12(2), 107-117.

Verma, V., Ravindran, P., Kumar, P.P. (2016). Plant hormone-mediated regulation of stress responses. BMC Plant Biology 16(86).

Vieites-Blanco, C., González-Prieto, S.J. (2018a) Effects of *Carpobrotus edulis* invasion on soil gross N fluxes in rocky coastal habitats. Science of the Total Environment 619-620, 966-976.

Vieites-Blanco, C., González-Prieto, S.J. (2018b). Effects of *Carpobrotus edulis* invasion on main litter and soil characteristics in backdune and rocky coastal habitats with oceanic climate. Plant Soil 425, 363-374.

Vieites-Blanco, C., Retuerto, R., Lema, M. (2019). Effects of the fungus *Sclerotinia sclerotiorum* and the scale insect *Pulvinariella mesembryanthemi* on the ice plant *Carpobrotus edulis* from native and non-native areas: Evaluation of the biocontrol potential. Biological Invasions 21, 2159-2176.

Vieites-Blanco, C., Rubén, R., Rodríguez, L., Rodríguez Lado, L., Lema, M. (2020). Potential distribution and population dynamics of *Pulvinariella mesembryanthemi*, a promising biocontrol agent of the invasive plant species *Carpobrotus edulis* and *C. aff. Acinaciformis.* Entomologia Generalis 40(2), 173-185.

Vilà, M., Weber E., D'Antonio C.M. (1998). Flowering and mating system in hybridizing *Carpobrotus* (Aizoaceae) in coastal California. Canadian Journal of Botany 76(7), 1165-1169.

Wallace, A.R. (1865). The action of natural selection in producing old age, decay, and death. A: Weismann, A. Eddays upon heredity and kindred biological problems. Oxford: Clarendon Press.

Wang, T., Hu, J.T., Wang, R.Q., Liu, C.H., Yu, D. (2018). Tolerance and resistance facilitate the invasion success of *Alternanthera philoxeroides* in disturbed habitats: A reconsideration of the disturbance hypothesis in the light of phenotypic variation. Environmental and Experimental Botany 153, 135-142.

Warren, C.R., Bleby, T., Adams, M.A. (2007). Changes in gas exchange versus leaf solutes as a means to cope with summer drought in *Eucalyptus marginata*. Oecologia 154(1), 1-10.

Weiher, E., Keddy, P.A. (1995). Assembly rules, null models, and trait dispersion—New questions front old patterns. Oikos 74(1), 159-164.

Weismann, A. (1889). Essays upon heredity and kindred biological problems. Oxford: Clarendon Press.

Weltzin, J.F., Belote, R.T., Sanders, N.J. (2003). Biological invaders in a greenhouse world: Will elevated CO_2 fuel plant invasions? Frontiers in Ecology and the Environment 1(3), 146-153.

Wesselingh, R.A., Klinkhamer, P.G.L., Jong, T.J., Boorman, L.A. (1997). Threshold size for flowering in different habitats: Effects of size-dependent growth and survival. Ecology 78(7), 2118-2132.

Williams, F., Eschen, R., Harris, A., Djeddour, D., Pratt, C., Shaw, R., et al. (2010). The economic cost of invasive non-native species on Great Britain. Wallingford: CABI.

Williams, G.C. (1957). Pleiotropy, natural selection and the evolution of senescence. Evolution 11(5), 398-411.

Williams, J.L. (2009). Flowering life-history strategies differ between the native and introduced ranges of a monocarpic perennial. American Naturalist 174(5), 660-672.

Williamson, M., Fitter, A.(1996) The varying success of invaders. Ecology 77(6),1661-66.

Williamson, M.H., Brown, K.C. (1986). The analysis and modelling of British invasions. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 314(1167), 505-522.

Wingler, A., Marès, M., Pourtau, N. (2004). Spatial patterns and metabolic regulation of photosynthetic parameters during leaf senescence. New Phytologist 161(3), 781-789.

Wu, S., Rejmánek, M., Grotkopp, E., Ditomaso, J.M. (2005). Herbarium records, actual distribution, and critical attributes of invasive plants: Genus *Crotalaria* in Taiwan. Taxon 54(1), 133-138.

Yamamoto, H.Y., Bassi, R. (1996). Carotenoids: Localization and function. A: Donald, O.R., Yocum, C.F. (eds) Oxygenic photosynthesis: The light reactions. Kluwer Academic Publishers, Dordrecht, 539-563

Zavaleta, E.S., Hobbs, R.J., Mooney, H.A. (2001). Viewing invasive species removal in a whole-ecosystem context. Trends in Ecology and evolution 16(8), 454-459.

Zhang, H., Sonnewald, U. (2017). Differences and commonalities of plant responses to single and combined stresses. The Plant Journal 90(5), 839-855.

Zunzunegui, M., Barradas, M.C.D., Ain-Lhout, F., Álvarez-Cansino, L., Esquivias M.P, et al. (2011). Seasonal physiological plasticity and recovery capacity after summer stress in Mediterranean scrub communities. Plant Ecology 212(1), 127-142.

Annexos

Annex 1. Marcadors de fotoprotecció i estrès fotooxidatiu com a eines útils per entendre l'èxit invasor

Annex 1. Photoprotection and photo-oxidative stress markers as useful tools to unravel plant invasion success.

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Chapter 9

Photoprotection and Photo-Oxidative Stress Markers As Useful Tools to Unravel Plant Invasion Success

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1 Introduction

Light is essential for life, but also potentially dangerous, particularly for plants. As sessile and photosynthetic organisms, plants benefit from solar irradiation but must also cope with it when too much light is received. The meaning of 'excess light' strictly refers to the amount of energy not used for photosynthesis in chloroplasts of plant leaves. A number of factors determine excess energy in chloroplasts, including not only the amount of solar radiation but also its quality and duration, the plant physiological status (including the development stage), plant stress tolerance, and the availability of other resources for plant growth (Demmig-Adams et al. 2017). As the name of "photosynthesis" itself reveals, light is the main resource for photosynthesis, this is, the conversion of light into chemical energy stored in carbohydrate molecules, synthesized from carbon dioxide and water, releasing oxygen. Under optimal conditions, light is captured by the light harvesting complexes (LHC) at the photosystems (PSI and PSII), which are found at the thylakoid membrane inside the chloroplast of the photosynthetic tissues (Croce and Van Amerongen 2011). Photosynthetic pigments, such as chlorophylls and carotenoids, are responsible of light capture and transference into reaction centres which ultimately allow redox reactions through the electron transport chain (ETC) leading to the ultimate reduction of NADP to NADPH. In addition, this creates a proton gradient across the chloroplast membrane, which is used by ATP synthase in the synthesis of ATP. The NADPH and ATP generated after the ETC are essential for carbon assimilation through their use in the Calvin cycle.

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There are different factors that can lead to suboptimal conditions for photosynthesis. For instance, low concentrations of the substrate for the Calvin cycle, i.e. CO_2, may lead to an accumulation of $NADPH^+$ at the ETC. A common plant response to stress is stomatal closure, which reduces water losses through transpiration but at the same time slows down the photosynthetic machinery. Besides low internal CO_2 concentration, high light itself may collapse the photosynthetic apparatus by an energy excess that cannot be used due to saturation on the ETC components. In PSII, a bound quinone (Q_A) receives the electron transferred from water splitting via the initial acceptor pheophytin. However, Q_A is not able to accept another electron from PSII until it has passed its electron to the next carrier, Q_B (Kalaji et al. 2014). In this state, the reaction centers are considered to be 'closed', leading to an accumulation of molecules of excited chlorophyll (^{3}Chl*). This, in turn, will inevitably cause a decline in quantum efficiency of PSII and damage on it due to the consequent generation of reactive oxygen species (ROS) (Apel and Hirt 2004). After damage on the PSII reaction centre by light excess, it must be dissembled and repaired. The D1 protein is the only compound that, when damaged, needs to be synthesized de novo (Goh et al. 2012). When the oxidation of D1 overcomes its regeneration capacity, photoinhibition occurs, thus leading to a light-induced reduction of the photosynthetic capacity (Takahashi and Badger 2011).

Not only at the PSII, but also at the PSI, the high energy received and the high tensions of oxygen found inside the chloroplast may lead to the formation of ROS, such as singlet oxygen ($^1O_2$), superoxide ion ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}$OH) (Asada 2006). Singlet oxygen is formed at the PSII due to an accumulation of excited chlorophylls (^{3}Chl*) (Havaux and Triantaphylides 2009). Singlet oxygen seems to be the major ROS involved in photo-oxidative stress-induced cell death, and is therefore a very interesting ROS to quantify, despite its high reactivity. The superoxide ion is formed at the PSI rapidly leading to hydrogen peroxide by the action of superoxide dismutase, potentially leading thereafter to the formation of hydroxyl radical, a very reactive ROS (Asada 2006). Here, we will use the term "photo-oxidative stress" as the imbalance between pro-oxidants (such as ROS) and antioxidant defences caused by excess energy in chloroplasts.

If photo-oxidative stress is not properly counterbalanced by antioxidant defences, oxidative damage occurs over different biomolecules, causing peroxidation of lipids, oxidation of proteins, and/or damaging nucleic acids. Photo-oxidative damage is therefore characterized by alterations of the membrane properties (changes in fluidity, ion transport), a loss of enzymatic activity, protein cross-linking, inhibition of protein synthesis, DNA damage and at the end, the death of the cells (Sharma et al. 2012). When this occurs irreversible by lack of sufficient regeneration capacity within cells and organs, this photo-oxidative damage in chloroplasts leads to photo-oxidative damage at the cellular, organ and eventually organism levels.

However, there are multiple photo-protective mechanisms that plants have developed to protect the chloroplast from photoinhibition and photo-oxidative stress (Takahashi and Badger 2011). These include from structural changes that reduce light collection, to an increase in the amounts of antioxidants that quench and/or scavenge ROS ("quenching" is considered here as the physical process eliminating ROS, while "scavenging" involves a chemical reaction for ROS elimination). All these responses reflect the plant's physiological status and correlate with different stresses intensity, being therefore highly informative to understand stress responses, compare genotypes and give insight into new alternatives to improve environmental management. Likewise, stress markers based on photo-oxidative stress may be helpful on some global ecological problems such as invasive plant species that constitute the second main threat to biodiversity. The utility of photo-oxidative stress markers in invasive plants studies lies in the fact that invasive vigour is determined by their physiological capacity overcoming the native coexistent species.

In this chapter, we aim at compiling existing information on the photoprotective and photo-oxidative stress markers used in plant invasion biology studies: from the study of the light harvesting complexes composition or the photosynthetic efficiency, to ROS formation and the accumulation of antioxidants and its oxidation. Much emphasis will be put on providing the essential information that each marker offers, but also their limitations and the actual and potential use in plant invasion studies.

2 Photoprotection and Photo-Oxidative Stress Markers: How to Measure Them

A **photo-oxidative stress marker** could be considered any molecule, ratio, index or general descriptor that responds to excess light and is related to oxidative stress. The different approaches to quantify photoprotection and photo-oxidative stress comprise the different defense levels that the plants trigger to respond to it and its measurement may include both in situ and ex situ measurements (Fig. 9.1). At the first level, the composition of light harvesting complexes regulates the light capture process at the thylakoid membrane (Walters 2005). Hence, plant pigments play a crucial role on the capacity to transfer light energy into the ETC that will ultimately lead into the production of ATP and storing the reducing power as NADPH. The efficiency by which the electrons are transferred can constitute also a stress marker, providing information on the actual degree of photoinhibition of the photosynthetic apparatus (Kalaji et al. 2014). If the energy exceeds the photosynthetic capacity, ROS are generated and, thus, an estimation of ROS production and/or the accumulation of antioxidants (preferably including their redox state) is another way to get a proxy of the extent of photo-oxidative stress. Finally, the accumulation of oxidation products is also a measure of the degree of oxidative stress. We are going to get through the different approaches to measure photoprotection and photo-oxidative stress and present the most used techniques (resumed in Table 9.1), taking into account what information we are really getting from them, including their limitations and requirements.

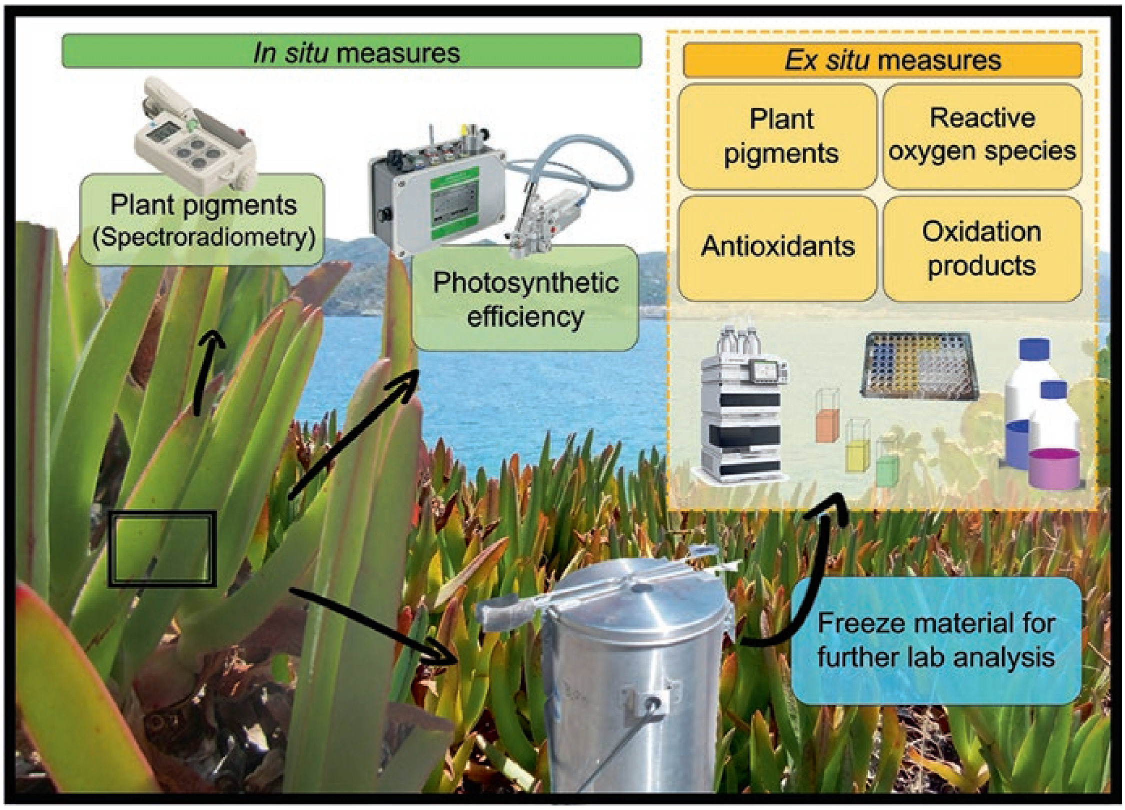

Fig. 9.1 Form field to lab in an invasion biology study, here exemplified with the aggressive invasive plant species *Carpobrotus edulis*, using photo-oxidative stress markers, measured in situ and ex situ. As examples, SPAD (MCL502, Minolta SPAD 502, GIS Ibérica, Spain), MiniPam II (Heinz WalzGmbH, Germany) and an Agilent HPLC are included here

2.1 Sampling Design

Before going through the different approaches and techniques used to measure photoprotection and photo-oxidative stress markers, it is important to point out some common requirements related to the sampling design.

At first, **representativeness** must be seriously considered when designing our experimental set, taking into account the high biological diversity at multiple levels. It is well known that biological diversity is wide, not only at the species level but also among different individuals from the same species, and even at the intraindividual level. For instance, there is an incredible variability considering the different organs within an individual taking into account the cellular structure and its biochemistry.

Choosing an appropriate number of replicates is essential to capture the biological variation, but it may depend on the study scale (growth chamber, common garden, field, ecosystem, international, etc.). In general, pseudo-replications are not recommended if we are after a real representation on the plant response to its environment. The number of replicates must increase after the variability on the environmental conditions and the differences among individuals (age, size, number and

Table 9.1 Summary of techniques and stress markers used to assess photoprotection and photo-oxidative stress in invasion biology and other ecophysiological studies, including a qualitative evaluation of their difficulty, accuracy, costs and dependency on other markers

	Photo-oxidative stress markers	Technique	Difficulty		Accuracy		Costs		Dependency on other markers
Plant pigments	Chl T	Spectrophotometry or HPLC	L	H	M	H	L	H	L
	Chl a/b	Spectrophotometry or HPLC	L	H	M	H	L	H	L
	Car/Chl	Spectrophotometry or HPLC	L	H	M	H	L	H	L
	VAZ	HPLC	H		H		H		L
	DPS	HPLC	H		H		H		L
	Lut	HPLC	H		H		H		H
	β-Car	HPLC	H		H		H		L
	Anthocyanins	Spectrophotometry	L		M		L		M
	SPAD	Spectroradiometry	L		L		L		H
	NDVI	Spectroradiometry	L		L		L		H
	PRI	Spectroradiometry	L		L		L		M
Photosynthetic efficiency	F_v/F_m	Fluorescence	L		H		L		L
	Φ_{PSII} or ETR	Fluorescence	L		H		L		L
	NPQ	Fluorescence	L		H		L		L
ROS	H_2O_2	Spectrophotometry	M		M		H		M
Antioxidants	AsA	Spectrophotometry	M		M		H		L
	AsA/(AsA+DHA)	Spectrophotometry	M		M		H		L
	α-, β-, γ-, δ-Toc	HPLC	H		H		H		L
Oxidation products	LOOH	Spectrophotometry	M		M		H		M
	MDA	Spectrophotometry or HPLC	M	H	M	H	M	H	M
	Protein carbonylation	Spectrophotometry or HPLC	M	H	M	H	M	H	H
	β-CC	GC/MS	H		H		H		H

This last parameter refers to the possibility of understanding photo-oxidative stress with the stress marker alone. The color code refers to the goodness of the qualification, from green (adequate) to orange (not adequate)
L low, *M* medium, *H* high

position of leaves, phenology, reproductive effort, etc.). For measures of photoprotection and photo-oxidative stress markers on ecophysiological studies performed under natural conditions, a number of replicates between 10 and 20 is in general recommended per sampling point, treatment and genotype, with a minimum of at least 8 individuals. The standard deviation can indeed be used as a measure of variation in each particular case.

To guarantee representativeness, some considerations of what and when we should sample are recommended, such as limit our sampling material to **leaves at the same developmental stage** and sample always under **similar environmental conditions**. Attention should be paid to avoid other factors influencing potential differences such as light incidence or biotic stress. We recommend sampling fully-expanded young leaves that receive direct solar radiation to minimize heterogeneity.

As photoprotection and photo-oxidative stress markers are strongly light-dependent, it is crucial to choose similar sampling environmental conditions and time of the day for measurements. We recommend performing **samplings during midday** (when the sun is at its zenith) on clear, sunny days.

Most of the techniques here presented require laboratory analysis (Fig. 9.1) and they need special **considerations to prevent sample degradation** and **additional measures** to estimate the final concentration. During sampling, samples must be immediately frozen in liquid nitrogen and stored at −80 °C until analysis to prevent sample degradation and changes on the cellular redox state. Moreover, as some of the molecules are highly reactive, thermo- or light sensitive, it is recommended to perform all analysis under cold conditions (4 °C) and protecting the samples against direct light. It is necessary to calculate the fresh weight/dry weight ratio of each sample and if possible the leaf mass area (LMA), to present the quantified molecules by fresh weight, dry weight and leaf area.

2.2 Plant Pigments

Among the Viridiplantae subkingdom (vascular plants, mosses and green algae), pigment composition has been shown to be remarkably constant, with chlorophylls *a* (Chl *a*) and *b* (Chl *b*) and six carotenoids: lutein (Lut), β-carotene (β-Car), neoxanthin (Neo), violaxanthin (Vio), antheraxanthin (Ant) and zeaxanthin (Zea), being found in all species (Young et al. 1997). Each of these pigments plays a specific role and is distinctively located within the photosynthetic apparatus (Croce and Van Amerongen 2011; Takahashi and Badger 2011). All these pigments play a dual role by collecting light through the light harvesting complexes (LHC) and offering photoprotection at the photosystem II, where light is initially collected.

Chlorophylls are the main photosynthetic pigments responsible for light capture, constituting therefore good photo-oxidative stress markers. Chlorophylls are found in cyanobacteria, algae and plants and are composed by a large heterocyclic aromatic ring with a magnesium ion at the centre of it. Chl *a* is present in the reaction centres and the antennae of PSI and PSII, whereas the presence of Chl *b* is restricted to light-harvesting systems (Croce and Van Amerongen 2011). Therefore, the ratio Chl *a/b* could be an indicator of the degree of sun/shade acclimation or the intensity of a stress (Esteban et al. 2015). Also, the content of total Chl itself respond to the widest variety of stressors (Esteban et al. 2015). Chlorophyll loss is a process associated with both intense stress and senescence processes (Zimmermann and Zentgraf 2005).

On the other hand, **carotenoids** belong to the category of tetraterpenoids, which take the form of a polyene hydrocarbon chain, which is sometimes terminated by rings. This group of isoprenoids play a dual role in the photosynthetic machinery, as light-harvesting pigments (Bontempo e Silva et al. 2012), but also they protect against photooxidative damage (Lambrev et al. 2012). This group is subdivided into carotenes, of which β-carotene (β-Car) is the most abundant, and xanthophylls, which contain oxygen in is chemical structure and include lutein, violaxanthin, zeaxanthin, antheraxanthin and neoxanthin. β-Car is especially efficient at eliminating the singlet oxygen ($^1O_2$) generated in photosystem II (PSII) from excited triplet chlorophyll (^{3}Chl*) (Ramel et al. 2012). Lutein is the most abundant xanthophyll species in plants and is essential for protein folding and ^{3}Chl* quenching (Dall'Osto et al. 2006). Moreover, xanthophylls are crucial as physical quenchers that promote thermal dissipation or non-photochemical quenching (NPQ), an efficient energy-dissipation mechanism in plants (Demmig-Adams and Adams 1996). The de-epoxidation of Vx to Ax and Zx (components of the VAZ cycle) responds to different environmental stresses (Demmig-Adams et al. 2012).

There are other plant pigments with an important role on photoprotection and widely distributed among the plant kingdom: **anthocyanins**, a class of flavonoids. These water-soluble pigments consist of an aromatic ring bound to a heterocyclic ring that contains oxygen, which is linked through a carbon-carbon bond to a third aromatic ring (forming the anthocyanidins), in some cases all bound to a sugar moiety (forming the corresponding anthocyanins) (Castañeda-Ovando et al. 2009). Anthocyanins are responsible of screening ultraviolet (UV) light and therefore constitute an important photoprotective defense for plants, as UV comprises 7–9% of the total solar radiation energy (Jansena et al. 1998), protecting plants from PSII damage (Takahashi et al. 2010). Anthocyanins are responsible for some of the colors on leaves, flowers, fruits and seeds, and are not localized on the chloroplast but accumulated in vacuoles, especially in the leaf epidermis cells, together with other phenolic compounds that accomplish the same screening photoprotective function (Takahashi and Badger 2011). The synthesis of phenolic compounds (including anthocyanins) is enhanced under strong light, particularly UV and blue light conditions (Winkel-Shirley 2002).

There are different techniques to measure plant pigments, all based on its specific light absorption spectrum (Table 9.1). With a liquid solvent (methanol, ethanol or acetone with different purity) we can easily extract all plant pigments. Calibration curves have been defined for determination of Chl *a*, Chl *b* and total carotenoids (Car) through **spectrophotometry** using different solvents (Lichtenthaler 1987). It was not until the late 1980s when good protocols for an easy and precise separation of the different carotenoids through **high-performance liquid chromatography** (HPLC), usually employing acetonitrile as the mobile phase, were developed (Thayer and Björkman 1990; Munné-Bosch and Alegre 2000). This is a relatively expensive approach, but it allows quantifying all the carotenes from one extract, offering the possibility to have a deeper understanding on the plant physiological status. Through this methodology, one can quantify how much energy the plant is dissipating through the xanthophyll cycle, by calculating the proportion of de-epoxidated xanthophylls, i.e. the de-epoxidation state ((DPS = (Zx + Ax)/Vx). Not only the DPS but the total amount of Vx, Ax and Zx (so called VAZ) increases in response to stress (Demmig-Adams and Adams 1996).

Anthocyanins can also be measured both by spectrophotometry and HPLC, taking advantage of the absorption range of the spectrum among 500–530 nm of these reddish pigments. There are several methods that show different specificity. At first, the most used method, due to its simplicity, is to estimate total anthocyanins by acidifying the methanol extract with 1% HCl and reading absorbance at 535 nm (Siegelman and Hendricks 1958; Fuleki and Francis 1968), always subtracting unspecific absorbance at 700 nm. Despite the simplicity of this method, it shows low specificity as all reddish pigments are quantified as anthocyanins, as phlobaphenes (Winkel-Shirley 2002). Another method is the pH differential method or the total monomeric anthocyanin method, designed to measure only single anthocyanin units (Giusti and Wrolstad 2001). Monomeric anthocyanins can change their colour under different acidic conditions, and the lectures at pH 1 and 4.5 comparison allows the removal of the interference of other reddish pigments, being an interesting method for several species (Lee et al. 2005; Dandena et al. 2011). Spectrophotometric methods usually use cyanidin-3-glucoside chloride as a standard, taking its extinction coefficient, as the main anthocyanin found in plants. HPLC methods can be used not only for a more precise quantification but also for identification of the precise anthocyanin composition. Different procedures have been proposed, including an acid hydrolysis that breaks the glyosidic bond of monomeric anthocyanins, releasing anthocyanidins (Lao and Giusti 2016).

Different indices and techniques based on **spectroradiometry** have been described also for chloroplastic pigments determination. Based on leaf transmittance, SPAD (MCL502, Minolta SPAD 502, GIS Ibérica, Spain) is a simple and portable apparatus that determines a relative quantity of chlorophylls by a simple non-destructive leaf measurement (Richardson et al. 2002) that can be measured in situ (Fig. 9.1). The relative measures show a high correlation with the total chlorophyll content and therefore it is a simple and fast alternative to laboratory analysis. However, this measure shows a high variability and needs calibration depending on the species and the environmental conditions. Based on leaf reflectance there are a whole plethora of different defined indexes with different applications. The broadest index used is the normalized difference vegetation index (NDVI), which is known for its good correlation with this chlorophyll content (Richardson et al. 2002). Another commonly used spectral reflectance index is the photochemical reflectance index (PRI) that often strongly correlates with total carotenoids or chlorophyll *a/b*, but also with radiation use efficiency, chlorophyll fluorescence parameters, DPS, net CO_2 uptake, Jmax or water content (Garbulsky et al. 2011). NDVI and PRI can be calculated at different scales, using different platforms where we use the spectroradiometer such as a drone, a balloon, planes or satellites. Spectroradiometric indexes are a promising tool for high-throughput phenotyping, but this technique requires calibration depending on the species, the season, the environmental conditions, etc., and normally there is a huge variability associated.

2.3 *Photosynthetic Efficiency*

Once a chlorophyll molecule gets excited, it helps the transference of an electron through the different proteinic complexes that form the electron transport chain (ETC). At the end, through the generation of an H^+ gradient, this process will generate ATP and accumulate reducing power as NADPH, necessary for Calvin cycle. An easy, in situ, and non-destructive way to measure the efficiency by which electrons pass through the ETC and detect photoinhibition is the measurement of **chlorophyll**

***a* fluorescence** (Fig. 9.1). The illumination of the photosynthetic tissue with photosynthetic active radiation leads to the emission of fluorescence (680–760 nm), mainly associated with chlorophyll *a* on the PSII. This fluorescence is one of the three ways where chlorophylls excitation energy is distributed, apart from the photochemical reactions on the ETC and the thermal dissipation (explained above). As the three processes are competitive, it is possible to estimate them from the chlorophyll fluorescence measurements. A range of instruments has been developed focusing on different aspects of photosynthesis and on different properties of Chl *a* fluorescence, but most authors are using only a limited set of experimental protocols based on methods that have been developed over time (Kalaji et al. 2014). One of the favorite techniques involves the use of a modulated measuring system, which allows the quantification of the contribution of the photochemical and non-photochemical quenching. In darkness, all the PSII reaction centers are open (all the quinone pool is reduced) and when a leaf is transferred from the darkness into light, PSII reaction centers close progressively. The comparison between the fluorescence emitted after a short duration saturation flash light (that immediately reduces the whole quinone pool) under natural light and after darkness adaptation allows the differentiation between the energy derived to photochemical and non-photochemical processes. In darkness, with all the reaction centers open, the increase on the fluorescence emission ($Fv = Fm - F_0$, variance, maximum and basal fluorescence) due to the saturation flash light indicates the maximum capacity of the PSII to transport electrons. One of the most widely used photo-oxidative stress markers is the maximum efficiency of the PSII (F_v/F_m), calculated from the parameters presented above, and measured by all the modulated fluorimeters. For unstressed leaves, the value of F_v/F_m is highly consistent, with values of ~0.83, and correlates to the maximum quantum yield of photosynthesis (Demmig and Björkman 1987). F_v/F_m below 0.75 reflect damage on the PSII, photoinhibition, and therefore it is an extremely informative stress marker.

More information can be obtained from chlorophyll *a* fluorescence analysis. The same calculation from the basal and maximum fluorescence after a saturating flash pulse under natural light gives the relative efficiency of the PSII (φ_{PSII}) and the electron transport rate (ETR). The latter requires the use of the average ratio of light absorbed by the leaf (around 0.84) and the average ratio of PSII reaction centers to PSI reaction centers (0.50) for calculation. Another parameter obtained from chlorophyll fluorescence analysis is NPQ. This parameter estimates the non-photochemical quenching and its calculation involves both light and dark-adapted measures. NPQ is calculated as $(Fm - Fm')/Fm'$, with the prima parameters corresponding to those taken under light conditions. NPQ strongly correlates with DPS (Demmig-Adams et al. 2012; Jahns and Holzwarth 2012), being a cheaper and non-destructive alternative to the measurement of the xanthophyll cycle by HPLC. The most attractive feature of Chl *a* fluorescence is its non-invasive character, but it is common to commit some pitfalls with the measures. Several reviews have elegantly compiled common pitfalls, questions and conflictive points of view of Chl *a* fluorescence techniques (Maxwell and Johnson 2000; Logan et al. 2007; Murchie and Lawson 2013; Kalaji et al. 2014).

2.4 Reactive Oxygen Species

We define oxidative stress as the imbalance between prooxidants and antioxidants. Therefore, the amount of reactive oxygen species (ROS), among other prooxidants, gives us information about the status of the imbalance during a stress response. Chloroplasts are quantitatively and qualitatively one of the most important sources of ROS in illuminated plant cells (Foyer and Noctor 2003). Thus, the measurement of singlet oxygen ($^1O_2$), superoxide ion ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) are good markers to evaluate the status of the photosynthetic apparatus.

There are three approaches for measuring ROS in plant tissues: (1) monitoring ROS released into a medium where the cell culture grows, (2) in vivo ROS visualization and (3) quantification of ROS production (Noctor et al. 2016). The third group is indeed the best suited for ecophysiology experiments. Here we will present the measures of hydrogen peroxide as it is the most stable of the group of the four primary ROS (H_2O_2, superoxide ion, hydroxyl radical and singlet oxygen), and therefore it is quantifiable after direct extraction (third approach).

Hydrogen peroxide (H_2O_2) can be quantified through spectral changes of different substances when they are oxidized by this molecule. For instance, the ferrous xylenol orange (FOX) assay is based on the oxidation of ferrous to ferric ions by H_2O_2 producing a chromophore complex which absorbs strongly at 540–600 nm (Cheeseman 2006); however, there are some matrix effects that may be taken into consideration (Queval et al. 2008). Another method is the use of Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) which is converted to the fluorescent resorufin, easily quantified with a fluorescence spectrophotometer (Zhou et al. 1997). For estimation of the extent of photo-oxidative stress, chloroplasts can be isolated from leaves under reducing conditions and the amount of ROS measured thereafter. This is essential for ROS that can be produced in various cellular compartments, as it occurs with hydrogen peroxide (Munné-Bosch et al. 2013).

2.5 Antioxidants

An antioxidant is a molecule that prevents the oxidation of other molecules. One of the most common responses to stress is the activation of antioxidant defences. We can classify the antioxidants into enzymatic and non-enzymatic, and the latter, depending on their affinity to water can be classified into hydrophilic and lipophilic antioxidants. The enzymatic antioxidants, (such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR)), among others) are found in chloroplasts, but also in other cellular compartments reducing oxidative stress. Therefore, they are not necessarily only related to photo-oxidative stress and specific chloroplastic isoforms should therefore be investigated to relate them to excess light energy. In contrast, a clear and strong relationship has been established between photo-oxidative stress and the accumulation and tocopherols (or vitamin E) and carotenoids (Car), since both are exclusively located in chloroplasts.

The group of **tocopherols (Toc)** include the α-, β-, γ-, and δ-tocopherols, which are differentiated by the number and position of methyl groups on the chromanol ring. It is specifically this chromanol head that provides the molecule its antioxidant scavenging properties as it can donate electrons to various acceptors such as $^{\cdot}OH$ or $^1O_2$. Tocopherols also deactivate singlet oxygen by (physical) quenching, being this latter function the most important quantitatively, protecting PSII from photo-oxidative damage. Tocopherols, which are located on the thylakoid membrane, but accumulate as well in the plastoglobuli (where they are stored), have also an essential role in preventing the propagation of lipid peroxidation (scavenging lipid peroxyl radicals, Munné-Bosch and Alegre 2002a). The contents of tocopherols, in agreement with their antioxidant function, increase in plants adapted to drought and other abiotic stresses (Munné-Bosch 2005). The four tocopherol homologues can be measured after an extraction with methanol by HPLC with a mixture of n-hexane and p-dioxane as a mobile phase, using a fluorescence detector, emitting at 330 nm and with detection at 295 nm (Amaral et al. 2005). The major homologue found in leaves is the α-tocopherol, followed by its immediate precursor, γ-tocopherol. β-, and δ-tocopherols are usually present at very low concentrations in leaves.

Ascorbic acid (AsA) is the most abundant hydrophilic antioxidant in plant leaves, and it is mainly accumulated in the chloroplast (Queval and Noctor 2007). Ascorbate can be oxidized to monodehydroascorbate radical (MDHA) or dehydroascorbate (DHA). Not only the total amount of AsA, but also the redox state of the ascorbic acid pool (AsA/(AsA + DHA)), particularly when measured in isolated chloroplasts, constitute excellent photo-oxidative stress markers and have been described to be very sensitive to several stresses. The most popular techniques for measuring AsA are based on the molecule's absorbance at 256 nm. To determine the amount of reduced and oxidised AsA is common to use reducing agents such as dithiothreitol (DTT) and ascorbate oxidase (AO), that reduce/oxide the whole sample extract in an acid medium and compare the maximum and the minimum absorbance with the initial one (Queval and Noctor 2007). AsA has an intimal relationship with tocopherols as it mediates their regeneration. At the same time, AsA is regenerated from DHA to AsA by **glutathione (GSH)**, another hydrophilic antioxidant found in most organelles.

2.6 Oxidation Products

As a result of photo-oxidative stress, if ROS are not counterbalanced by antioxidant defences, oxidative damage occurs over different biomolecules. The derived products of this process: oxidized compounds, such as primary or secondary lipid peroxidation products and modified proteins constitute excellent photo-oxidative stress markers focusing on the consequences after the damage. The enhanced production of ROS during environmental stresses can pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, and/or damage to nucleic acids, thus causing enzyme inhibition, alterations of the membrane properties (changes in fluidity, ion transport), protein cross-linking, inhibition of protein synthesis, DNA damage and at the end, the death of the cells (Sharma et al. 2012).

Over lipids, free radicals or ROS can inflict direct damage, leading to lipid peroxidation that at the same time can inflict damage over DNA or the protein complexes of the PSII (Pospísil and Yamamoto 2017). This is the process under which free radicals attack polyunsaturated fatty acids (PUFAs) of the phospholipidic membrane from the cell or its organelles, essential for cell survival (Ayala and Muñoz 2014). Hydroxyl radical ($HO^{\cdot}$) and hydroperoxyl ($HO^{\cdot}_2$) are the most dangerous ROS for lipids, and a single molecule of ROS can result in multiple peroxided PUFAs as they trigger a cyclic chain reaction that propagates itself very fast (Sharma et al. 2012). The overall process of lipid peroxidation consists of three steps: initiation, propagation and termination (Schneider 2009). During initiation, ROS react with methylene groups of PUFA forming lipid peroxy radicals and hydroperoxides (LOOH). These lipidic products formed are highly reactive and attack other lipids propagating the chain reaction at the propagation phase. After that reactions several reactive species including lipid alkoxyl radicals, aldehydes (malonyldialdehyde, among others), alkanes, lipid epoxides and alcohols are formed by the decomposition of lipid hydroperoxides (Davies 2000). In the termination phase, antioxidants such as vitamin E donate a hydrogen atom to the lipid peroxyl radical ($LOO^{\cdot}$) species forming tocopheroxyl radical that reacts with another $LOO^{\cdot}$ forming nonradical products (Ayala and Muñoz 2014).

Proteins can be affected directly or indirectly by ROS. Direct modifications include modification of its activity trough nitrosylation, carbonylation, disulphide bond formation and glutathionylation, while indirect effects include protein conjugation with lipid peroxidation products (Sharma et al. 2012). Protein carbonylation is defined as an irreversible post-transcriptional modification that yields a reactive carbonyl moiety in a protein, such as an aldehyde or ketone (Fedorova et al. 2014). This is the most common protein modification derived from the oxidation by a ROS, and an enhanced modification of proteins has been reported in plants under various stresses, therefore considering it a major hallmark of oxidative stress (Dalle-Donne et al. 2006). The accumulation of carbonylated proteins results in biomolecule malfunctions that can lead to cell death (Curtis et al. 2013).

Reactive oxygen species, specially $^{\cdot}OH$ and $^1O_2$ constitute the main source of DNA damage resulting in deoxyribose oxidation, strand breakage, removal of nucleotides and a variety of modifications in the organic bases of the nucleotides (Sharma et al. 2012). Despite the fact that ROS can inflict damage to nuclear, mitochondrial and chloroplast DNA, the two last are more susceptible to oxidative damage than nuclear DNA, due to the lack of protective protein, histones, and because they are very close to locations where ROS is produced (Manova and Gruszka 2015).

Among the different biomolecules damaged by ROS, some are better suited than others to become good stress markers. For lipid damage, the accumulation of lipid peroxides or the secondary product malondialdehyde constitute good markers of lipid peroxidation. Protein carbonylation is being also used as a good marker of oxidative stress (Levine et al. 1994). However, neither lipid peroxidation nor protein carbonylation are exclusively formed in chloroplasts and their use as markers of photo-oxidative stress should be interpreted carefully. Protein carbonyls are in turn more stable (in a scale of hours/days) than lipid peroxidation products, which are removed within minutes (Weber et al. 2015).

The accumulation of **lipid peroxides** (LOOH) are key indicators of the degree of lipid peroxidation, and constitute a good stress marker (Niki 2014). There are multiple approaches to measure the accumulation of LOOH. For plant samples, an easy method can be performed after a methanol extraction through **spectrophotometry** using again the FOX method, which measures the oxidation from ferrous to ferric ions by LOOH, in comparison with an extract where all LOOH are reduced by adding triphenylphospine (TPP). The ferric ions form a chromophore complex with the xylenol orange that absorbs at 540–600 nm (Bou et al. 2008).

Malondialdehyde (MDA) is one of the oxidation products derived from lipid peroxidation and usually measured by several studies assessing the degree of oxidative stress (see some examples in Table 9.2). The assay of thiobarbituric acid-reactive substances (TBARS) is the most used method to assess the breakdown products from lipid peroxidation, including MDA. The TBARS assay includes a liquid extraction with 80% ethanol and measure at 440, 532 and 600 nm with the **spectrophotometer** (Du and Bramlage 1992; Hodges et al. 1999) after an incubation with thiobarbituric acid. Higher precision can be obtained by **HPLC**, using a similar procedure (Iturbe-Ormaetxe et al. 1998; Munné-Bosch and Alegre 2002a).

3 Photoprotection and Photo-Oxidative Stress Markers in Invasion Biology Studies

The economical, demographic and technological development has allowed us to access to almost every biome causing some impacts, altering ecosystems functions. Moreover, globalization has led to the possibility that some species move along with humans, jumping off the geographical barriers that define the realized niche of each species, impacting on native ecosystems by changes on function and composition. Indeed, invasive species are considered the second major threat for the global biodiversity, after habitat loss (Simberloff et al. 2013). Invaders are supposed to have an increased vigor and/or an increased phenotypic plasticity underlying its ability to displace native species (Higgins and Richardson 2014). Therefore, invasive species may have increased physiological performance responding better to the environmental local conditions. In that way, the use of photoprotection and photo-oxidative stress markers may be helpful on invasive studies allowing a better comprehension of the boundaries of the physiological niche by understanding their stress tolerance and adaptation.

Photoprotection and photo-oxidative stress markers may be useful in invasion studies to understand the differences that may lead invasive species to outcompete natives through the description of their capacities under different environmental conditions and the affectation over native species. The comparison of invasive

Table 9.2 Compilation of the plant invasion studies using photoprotection and photo-oxidative stress markers during the last decade (since 2007)

Measurement	Methodology or type	References	Number of studies
Plant pigments	Spectro-photometry	Kim et al. (2008), Li et al. (2008), Liu et al. (2008), Mateos-Naranjo et al. (2008, 2010), Qaderi and Reid (2008), Qaderi et al. (2008), Zhang and Wen (2008), Feng (2008), Feng and Fu (2008), Funk (2008), Andrews et al. (2009), Hussner and Meyer (2009), Küpper et al. (2009), Yang et al. (2009), Feng et al. (2009), Zheng et al. (2012), Kaur et al. (2013), Funk et al. (2013), Castillo et al. (2014), Oliveira et al. (2014), Díaz-Barradas et al. (2015), Al Hassan et al. (2016), Huangfu et al. (2016), Lechuga-Lago et al. (2016), Lyu et al. (2016), Zhang et al. (2016), González-Teuber et al. (2017), Rotini et al. (2017), Souza-Alonso and González (2017), Varone et al. (2017), and Choi et al. (2017)	32
	HPLC	Cela et al. (2009), Song et al. (2010), Cela and Munné-Bosch (2012), Molina-Montenegro et al. (2012), Fleta-Soriano et al. (2015), Lassouane et al. (2016), Fenollosa et al. (2017), and Pintó-Marijuan et al. (2017)	8
	Spectro-radiometry	Ge et al. (2008), Spencer et al. (2008), Hestir et al. (2008), Funk and Zachary (2010), Naumann et al. (2010), Godoy et al. (2011), Roiloa et al. (2013, 2014, 2016), Wang et al. (2016), Yu et al. (2016), Heberling and Fridley (2016), and Roiloa and Retuerto (2016)	13
Photosynthetic efficiency	F_v/F_m only	Wang et al. (2008), Li et al. (2008), Liu et al. (2008), Bihmidine et al. (2009); Naumann et al. (2010); Funk and Zachary (2010), Redondo-Gómez et al. (2011), Immel et al. (2011), Waring and Maricle (2012), Roiloa et al. (2014, 2016), Madawala et al. (2014), Díaz-Barradas et al. (2015), Fleta-Soriano et al. (2015), Lechuga-Lago et al. (2016), Lyu et al. (2016), and Souza-Alonso and González (2017)	17
	F_v/F_m, NPQ, ΦPSII	Qaderi and Reid (2008), Richards et al. (2008), Zhang and Wen (2008), Funk (2008), Mateos-Naranjo et al. (2008. 2010), Cela et al. (2009), Wu et al. (2009), Yang et al. (2009), Song et al. (2010), Cela and Munné-Bosch (2012), Molina-Montenegro et al. (2012, 2016), Roiloa et al. (2013), Funk et al. (2013), Quinet et al. (2015), Li et al. (2015), Roiloa and Retuerto (2016), Lassouane et al. (2016), Pintó-Marijuan et al. (2017), Varone et al. (2017), Fenollosa et al. (2017), and Lukatkin et al. (2017)	23
ROS	H_2O_2	Kaur et al. (2013), Oliveira et al. (2014), and Mamik and Sharma (2017)	3

(continued)

Table 9.2 (continued)

Measurement	Methodology or type	References	Number of studies
Antioxidants	Enzymatic	Lu et al. (2007); Zhang and Wen (2008); Li et al. (2008); Immel et al. (2011), Redondo-Gómez et al. (2011), Morais et al. (2012), Huang et al. (2013), Kaur et al. (2013), Oliveira et al. (2014), Al Hassan et al. (2016), Zhang et al. (2016), and Mamik and Sharma (2017)	12
	Non-enzymatic	Cela et al. (2009), Cela and Munné-Bosch (2012), Huang et al. (2013), Fleta-Soriano et al. (2015), Al Hassan et al. (2016), and Pintó-Marijuan et al. (2017)	6
Oxidation products	MDA	Lu et al. (2007) ,(2008), Zhang and Wen 2008, Li et al. (2008), Immel et al. (2011), Falleh et al. (2012), Huang et al. (2013), Kaur et al. (2013), Oliveira et al. (2014), Quinet et al. (2015), Fleta-Soriano et al. (2015), Al Hassan et al. (2016), Molina-Montenegro et al. (2016), Zhang et al. (2016), Lassouane et al. (2016), and Mamik and Sharma (2017)	16

Measures of carotenoids are included on "Plant pigments" despite some of them have a role as antioxidants. Also, due to the high number of studies measuring the total amount of phenolic compounds, they have not been considered here inside the antioxidants group, although they are non-enzymatic antioxidants

species with coexistent natives had led to the conclusions that invaders have higher capacities to respond to stress or that they have broader physiological niches. Moreover, photo-oxidative stress markers can be helpful to predict plant responses to new environmental conditions, such as climate change. The direction of the community changes due to a new climatic framework can only be predicted with a complete ecophysiological approach. Finally, it is important to describe the extend of the differences between the genotypes from the invasive and the native ranges of one species. An in-depth understanding of these differences with the use of physiological descriptors (such as photo-oxidative stress markers) may undoubtedly help predict new invasions.

Although the interest to study the invasion process **using a complete ecophysiological approach** has increased recently, studies considering in-depth physiological processes are still limited (Pintó-Marijuan and Munné-Bosch 2013). However, photo-oxidative stress markers are being used more and more, and constitute indeed a promising tool for a better understanding of the invasion process. As we can see in Table 9.2, the most common photo-oxidative stress markers measured in plant invasion studies are photosynthetic pigments and chlorophyll *a* fluorescence parameters.

Considering the methodologies used, the most common measurements are photosynthetic pigments through spectrophotometry and the measure only of F_v/F_m. Indeed, only a few studies on the last decade include different measurements of photo-oxidative stress markers, which guarantee a complete understanding of the plant response. The most common combination is the measurement of photosynthetic pigments through spectrophotometry, the F_v/F_m, and the extend of lipid peroxidation through MDA analysis.

4 Some Limitations and Perspectives

The techniques to measure photo-oxidative stress markers present some common limitations. First, as Pintó-Marijuan and Munné-Bosch (2014) pointed out, it is very difficult to differentiate photo-oxidative damage caused by stress from that caused by **leaf senescence**. Senescence is the physiological deterioration with aging, and some of the hallmarks of senescence are chlorophyll loss and an increase of oxidative stress (Munné-Bosch and Alegre 2002b). Sampling fully-expanded young leaves throughout the experiment is the only way to separate stress- vs. senescence-related effects.

Another point to consider is the **localization** of the measured compound. For example, the measurement of ascorbic acid in leaves. A significant percentage of the ascorbic acid is normally found outside the chloroplast and an increase of this antioxidant can be a consequence of other processes rather than photo-oxidative stress. An easy (but time-consuming) way to ensure that we are measuring a photo-oxidative stress marker is to isolate chloroplasts.

The **matrix effect** must be cheeked every time we work with a new species or a known species under different conditions. Some of the protocols and the authors describing them indeed propose some alternatives to reduce matrix effects. One must keep in mind that some protocols are pH-dependent and the sample pH will depend on the species and its conditions.

It is essential to understand what **information** do we get from each photo-oxidative stress marker, and be aware of the fact that depending on the stress intensity we will see changes on different markers. Sometimes a **combined approach** with different photo-oxidative stress markers would be the most appropriate solution and the selection must follow the question we are trying to answer with our experiment.

As said before, there is a need to use combined stress markers. Photo-oxidative stress is a final consequence of the imbalance of different processes. It is not until antioxidant systems have been taken down that we can measure an accumulation of reactive oxygen species or oxidation products. Therefore, combined markers provide complementary information about the stress response. Here we propose some tips to perform a multiple approach to understand the global plant response.

If we focus on the obtained information, it would be ideal to take one photo-oxidative stress marker from the following groups: plant pigments, photosynthetic efficiency, reactive oxygen species, antioxidants and oxidation products. However, that represents multiple assays and a large amount of samples. If a faster and efficient protocol is needed, it is possible to connect different protocols. For instance, the extraction of plant pigments (chlorophylls, carotenoids and anthocyanins), tocopherols and lipid hydroperoxides have a common start, and all molecules can be extracted with methanol. Thus, it is possible to save time by performing a common extraction. If the limitation is the economy we can use the cheapest techniques, such as chlorophyll *a* fluorescence and spectroradiometric indexes, such as NDVI or PRI, which with the appropriate models can estimate some photo-oxidative stress markers (Table 9.1). The same techniques are useful if we have a high-scale experimental design (e.g. for large-scale phenotyping).

5 General Conclusions

Photoprotection and photo-oxidative stress are central elements of plant responses to a variety of stresses. Markers based on photoprotection and photo-oxidative stress may be extremely useful for understanding plant acclimation, and constitute a promising tool for the study of invasion success. Working with photoprotection and photo-oxidative stress markers requires the understanding of the meaning of every specific marker within the whole framework of the photoprotective mechanisms. As discussed in this chapter, a combined approach is required to better understand the ecophysiology of invasive vs. native species, using several markers providing complementary information. Here, we have provided some essential tools for a correct choosing of the fittest photoprotection and photo-oxidative stress markers, encouraging its use on invasion studies to help unravelling invaders success.

References

Al Hassan M, Chaura J, López-Gresa MP, Borsai O, Daniso E, Donat-Torres MP, Mayoral O, Vicente O, Boscaiu M (2016) Native-invasive plants vs. halophytes in mediterranean salt marshes: stress tolerance mechanisms in two related species. Front Plant Sci 7:1–21

Amaral JS, Casal S, Torres D, Seabra RM, Oliveira BPP (2005) Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method. Anal Sci 21:1545–1548

Andrews M, Hamish GM, Hodge S, Cherrill A, Raven JA (2009) Seed dormancy, nitrogen nutrition and shade acclimation of *Impatiens glandulifera*: implications for successful invasion of deciduous woodland. Plant Ecol Divers 2:145–153

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Ayala A, Muñoz MF (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Med Cell Longev 2014:360438

Bihmidine S, Bryan NM, Payne KR, Parde MR, Okalebo JA, Cooperstein SE, Awada T (2009) Photosynthetic performance of invasive *Pinus ponderosa* and *Juniperus virginiana* seedlings under gradual soil water depletion. Plant Biol 12:668–675

Bontempo e Silva EA, Hasegawa SF, Ono K, Sumida A, Uemura S, Hara T (2012) Differential photosynthetic characteristics between seedlings and saplings of *Abies sachalinensis* and *Picea glehnii*, in the field. Ecol Res 27:933–943

Bou R, Codony R, Tres A, Decker EA, Guardiola F (2008) Determination of hydroperoxides in foods and biological samples by the ferrous oxidation-xylenol orange method: a review of the factors that influence the method's performance. Anal Biochem 377:1–15

Castañeda-Ovando A, Pacheco-Hernández MDL, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA (2009) Chemical studies of anthocyanins: a review. Food Chem 113:859–871

Castillo JM, Grewell BJ, Pickart A, Bortolus A, Peña C, Figueroa E, Sytsma M (2014) Phenotypic plasticity of invasive S*partina densiflora* (Poaceae) along a broad latitudinal gradient on the pacific coast of North America. Am J Bot 101:448–458

Cela J, Munné-Bosch S (2012) Acclimation to high salinity in the invasive CAM plant *Aptenia cordifolia*. Plant Ecol Divers 53:403–410

Cela J, Arrom L, Munné-Bosch S (2009) Diurnal changes in photosystem II photochemistry, photoprotective compounds and stress-related phytohormones in the CAM plant, *Aptenia cordifolia*. Plant Sci 177:404–410

Cheeseman JM (2006) Hydrogen peroxide concentrations in leaves under natural conditions. J Exp Bot 57:2435–2444

Choi D, Watanabe Y, Guy RD, Sugai T, Toda H, Koike T (2017) Photosynthetic characteristics and nitrogen allocation in the black locust (*Robinia pseudoacacia* L.) grown in a FACE system. Acta Physiol Plant 39:1–12

Croce R, Van Amerongen H (2011) Light-harvesting and structural organization of Photosystem II: from individual complexes to thylakoid membrane. J Photoch Photobio B 104:142–153

Curtis JM, Hahn WS, Long EK, Burrill JS, Arriaga EA, Bernlohr DA (2013) Protein carbonylation and metabolic control systems. Trends Endocrin Met 23:399–406

Dall'Osto L, Lico C, Alric J, Giuliano G, Havaux M, Bassi R (2006) Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection in vivo under strong light. BMC Plant Biol 6:32

Dalle-Donne I, Aldini G, Carini M, Colombo R, Rossi R, Milzani A (2006) Protein carbonylation, cellular dysfunction, and disease progression. J Cell Mol Med 10:389–406

Dandena A, Leimane I, Kletnieks U (2011) Validation of monomeric anthocianin determination method for bilberry juice and marc extracts. J Life Sci 6:1378–1382

Davies KJ (2000) Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50:279–289

Demmig B, Björkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. Planta 171:171–184

Demmig-Adams B, Adams W (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci 1:21–26

Demmig-Adams B, Cohu CM, Muller O, Adams WW (2012) Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. Photosynth Res 113:75–88

Demmig-Adams B, Stewart JJ, Iii WWA (2017) Environmental regulation of intrinsic photosynthetic capacity: an integrated view. Curr Opin Plant Biol 37:34–41

Díaz-Barradas MC, Zunzunegui M, Álvarez-Cansino L, Esquivias MP, Collantes MB, Cipriotti PA (2015) Species-specific effects of the invasive *Hieracium pilosella* in Magellanic steppe grasslands are driven by nitrogen cycle changes. Plant Soil 397:175–187

Du Z, Bramlage WJ (1992) Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J Agric Food Chem 40:1566–1570

Esteban R, Barrutia O, Artetxe U, Fernández-Marín B, Hernández A, García-Plazaola JI (2015) Internal and external factors affecting photosynthetic pigment composition in plants: a meta-analytical approach. New Phytol 206:268–280

Falleh H, Jalleli I, Ksouri R, Boulaaba M, Guyot S, Magné C (2012) Effect of salt treatment on phenolic compounds and antioxidant activity of two *Mesembryanthemum edule* provenances. Plant Physiol Biochem 52:1–8

Fedorova M, Bollineni RC, Hoffmann R (2014) Protein carbonylation as a major hallmark of oxidative damage: update of analytical strategies. Mass Spectrom Rev 33:79–97

Feng YL (2008) Photosynthesis, nitrogen allocation and specific leaf area in invasive *Eupatorium adenophorum* and native *Eupatorium japonicum* grown at different irradiances. Physiol Plantarum 133:318–326

Feng YL, Fu GL (2008) Nitrogen allocation, partitioning and use efficiency in three invasive plant species in comparison with their native congeners. Biol Invasions 10:891–902

Feng Y-L, Lei Y-B, Wang R-F, Callaway RM, Valiente-Banuet A, Inderjit LY-P, Zheng Y-L (2009) Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant. Proc Natl Acad Sci U S A 106:1853–1856

Fenollosa E, Munné-Bosch S, Pintó-Marijuan M (2017) Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*. Physiol Plantarum 160:185–200

Fleta-Soriano E, Pintó-Marijuan M, Munné-Bosch S (2015) Evidence of drought stress memory in the facultative cam, *Aptenia cordifolia*: possible role of phytohormones. PLoS One 10:e0135391

Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plantarum 119:355–364

Fuleki T, Francis FJ (1968) Quantitative methods for anthocyanins. J Food Sci 33:72–77

Funk JL (2008) Differences in plasticity between invasive and native plants from a low resource environment. J Ecol 96:1162–1173

Funk JL, Zachary ÆVA (2010) Physiological responses to short-term water and light stress in native and invasive plant species in southern California. Biol Invasions 12:1685–1694

Funk JL, Glenwinkel LA, Sack L (2013) Differential allocation to photosynthetic and non-photosynthetic nitrogen fractions among native and invasive species. PLoS One 8:e64502

Garbulsky MF, Peñuelas J, Gamon J, Inoue Y, Filella I (2011) The photochemical reflectance index (PRI) and the remote sensing of leaf, canopy and ecosystem radiation use efficiencies. A review and meta-analysis. Remote Sens Environ 115:281–297

Ge S, Carruthers RI, Spencer DF, Yu Q (2008) Canopy assessment of biochemical features by ground-based hyperspectral data for an invasive species, giant reed (*Arundo donax*). Environ Monit Assess 147:271–278

Giusti MM, Wrolstad RE (2001) Characterization and measurement of anthocyanins by UV visible spectroscopy. CPFAC F1.2.1-F1.2.13

Godoy O, Saldaña A, Fuentes N, Valladares F, Gianoli E (2011) Forests are not immune to plant invasions: phenotypic plasticity and local adaptation allow *Prunella vulgaris* to colonize a temperate evergreen rainforest. Biol Invasions 13:1615–1625

Goh CH, Ko SM, Koh S, Kim YJ, Bae HJ (2012) Photosynthesis and environments: photoinhibition and repair mechanisms in plants. J Plant Biol 55:93–101

González-Teuber M, Quiroz CL, Concha-Bloomfield I, Cavieres LA (2017) Enhanced fitness and greater herbivore resistance: implications for dandelion invasion in an alpine habitat. Biol Invasions 19:647–653

Havaux M, Triantaphylides C (2009) Singlet oxygen in plants: production, detoxification and signaling. Trends Plant Sci 14:219–228

Heberling JM, Fridley JD (2016) Invaders do not require high resource levels to maintain physiological advantages in a temperate deciduous forest. Ecology 97:874–884

Hestir EL, Khanna S, Andrew ME, Santos MJ, Viers JH, Greenberg JA, Rajapakse SS, Ustin SL (2008) Identification of invasive vegetation using hyperspectral remote sensing in the California Delta ecosystem. Remote Sens Environ 112:4034–4047

Higgins SI, Richardson DM (2014) Invasive plants have broader physiological niches. Proc Natl Acad Sci U S A 111:10610–10614

Hodges DM, DeLong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604–611

Huang Y, Bai Y, Wang Y, Kong H (2013) Allelopathic effects of the extracts from an invasive species *Solidago canadensis* L. on *Microcystis aeruginosa*. Lett Appl Microbiol 57:451–458

Huangfu C, Li H, Chen X, Liu H, Wang H, Yang D (2016) Response of an invasive plant, *Flaveria bidentis*, to nitrogen addition: a test of form-preference uptake. Biol Invasions 18:3365–3380

Hussner A, Meyer C (2009) The influence of water level on the growth and photosynthesis of *Hydrocotyle ranunculoides* L.fil. Flora 204:755–761

Immel F, Renaut J, Masfaraud J (2011) Physiological response and differential leaf proteome pattern in the European invasive Asteraceae *Solidago canadensis* colonizing a former cokery soil. J Proteome 75:1129–1143

Iturbe-Ormaetxe I, Escuredo PR, Arrese-Igor C, Becana M (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol 116:173–181

Jahns P, Holzwarth AR (2012) The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. BBA-Bioenergetics 1817:182–193

Jansena MAK, Gaba V, Greenberg BM (1998) Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci 3:131–135

Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, Brestic M et al (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynth Res 122:121–158

Kaur T, Bhat HA, Raina A, Koul S, Vyas D (2013) Glutathione regulates enzymatic antioxidant defence with differential thiol content in perennial pepperweed and helps adapting to extreme environment. Acta Physiol Plant 35:2501–2511

Kim YO, Rodriguez RJ, Lee EJ, Redman RS (2008) *Phytolacca americana* from contaminated and noncontaminated soils of South Korea: effects of elevated temperature, CO_2 and simulated acid rain on plant growth response. J Chem Ecol 34:1501–1509

Küpper H, Götz B, Mijovilovich A, Küpper FC, Meyer-Klaucke W (2009) Complexation and toxicity of copper in higher plants: characterization of copper accumulation, speciation, and toxicity in *Crassula helmsii* as a new copper accumulator. Plant Physiol 151:702–714

Lambrev PH, Miloslavina Y, Jahns P, Holzwarth AR (2012) On the relationship between non-photochemical quenching and photoprotection of Photosystem II. Biochim Biophys Acta 1817:760–769

Lao F, Giusti MM (2016) quantification of purple corn (*Zea mays* L.) anthocyanins using spectrophotometric and HPLC approaches: method comparison and correlation. Food Anal Methods 9:1367–1380

Lassouane N, Aïd F, Lutts S (2016) Drought inhibits early seedling establishment of *Parkinsonia aculeata* L. under low light intensity: a physiological approach. Plant Growth Regul 80:115–126

Lechuga-Lago Y, Sixto-Ruiz M, Roiloa SR, González L (2016) Clonal integration facilitates the colonization of drought environments by plant invaders. AoB Plants 8:plw023

Lee J, Durst RW, Wrolstad RE (2005) Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int 88:1269–1278

Levine RL, Williams JA, Stadtman EP, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Method Enzymol 233:346–357

Li H, Qiang S, Qian Y (2008) Physiological response of different croftonweed (*Eupatorium adenophorum*) populations to low temperature. Weed Sci 56:196–202

Li W, Luo J, Tian X, Soon Chow W, Sun Z, Zhang T, Peng S, Peng C (2015) A new strategy for controlling invasive weeds: selecting valuable native plants to defeat them. Sci Rep 5:11004

Lichtenthaler HK (1987) Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. Plant Phys 131:101–110

Liu J, He WM, Zhang SM, Liu FH, Dong M, Wang RQ (2008) Effects of clonal integration on photosynthesis of the invasive clonal plant *Alternanthera philoxeroides*. Photosynthetica 46:299–302

Logan BA, Adams WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. Funct Plant Biol 34:853–859

Lu P, Sang WG, Ma KP (2007) Activity of stress-related antioxidative enzymes in the invasive plant crofton weed (*Eupatorium adenophorum*). J Integr Plant Biol 49:1555–1564

Lu P, Sang WG, Ma KP (2008) Differential responses of the activities of antioxidant enzymes to thermal stresses between two invasive *Eupatorium* species in China. J Integr Plant Biol 50:393–401

Lukatkin AS, Tyutyaev EV, Sharkaeva ES, Lukatkin AA, Teixeira da Silva JA (2017) Mild abiotic stresses have different effects on chlorophyll fluorescence parameters in leaves of young woody and herbaceous invasive plants. Acta Physiol Plant 39:1–7

Lyu XQ, Zhang YL, You WH (2016) Growth and physiological responses of *Eichhornia crassipes* to clonal integration under experimental defoliation. Aquat Ecol 50:153–162

Madawala S, Hartley S, Gould KS (2014) Comparative growth and photosynthetic responses of native and adventive iceplant taxa to salinity stress. New Zeal J Bot 52:37–41

Mamik S, Sharma AD (2017) Analysis of boiling stable antioxidant enzymes in invasive alien species of *Lantana* under abiotic stress-like conditions. Rev Bras Bot 37:129–141

Manova V, Gruszka D (2015) DNA damage and repair in plants – from models to crops. Front Plant Sci 6:1–26

Mateos-Naranjo E, Redondo-Gómez S, Cambrollé J, Luque T, Figueroa ME (2008) Growth and photosynthetic responses to zinc stress of an invasive cordgrass, *Spartina densiflora*. Plant Biol 10:754–762

Mateos-Naranjo E, Redondo-Gómez S, Álvarez R, Cambrollé J, Gandullo J, Figueroa ME (2010) Synergic effect of salinity and CO_2 enrichment on growth and photosynthetic responses of the invasive cordgrass *Spartina densiflora*. J Exp Bot 61:1643–1654

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. J Exp Bot 51:659–668

Molina-Montenegro MA, Peñuelas J, Munné-Bosch S, Sardans J (2012) Higher plasticity in ecophysiological traits enhances the performance and invasion success of *Taraxacum officinale* (dandelion) in alpine environments. Biol Invasions 14:21–33

Molina-Montenegro MA, Galleguillos C, Oses R, Acuña-Rodríguez IS, Lavín P, Gallardo-Cerda J, Torres-Díaz C, Diez B, Pizarro GE, Atala C (2016) Adaptive phenotypic plasticity and competitive ability deployed under a climate change scenario may promote the invasion of *Poa annua* in Antarctica. Biol Invasions 18:603–618

Morais MC, Panuccio MR, Muscolo A, Freitas H (2012) Salt tolerance traits increase the invasive success of *Acacia longifolia* in Portuguese coastal dunes. Plant Physiol Biochem 55:60–65

Munné-Bosch S (2005) The role of alpha-tocopherol in plant stress tolerance. Plant Physiol 162:743–748

Munné-Bosch S, Alegre L (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210:925–931

Munné-Bosch S, Alegre L (2002a) Interplay between ascorbic acid and lipophilic antioxidant defences in chloroplasts of water-stressed Arabidopsis plants. FEBS Lett 524:145–148

Munné-Bosch S, Alegre L (2002b) Plant aging increases oxidative stress in chloroplasts. Planta 214:608–615

Munné-Bosch S, Queval G, Foyer CH (2013) The impact of global change factors on redox signalling underpinning stress tolerance. Plant Physiol 161:5–19

Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Bot 64:3983–3998

Naumann JC, Bissett SN, Anderson JE (2010) Diurnal patterns of photosynthesis, chlorophyll fluorescence, and PRI to evaluate water stress in the invasive species, *Elaeagnus umbellata* Thunb. Trees 24:237–245

Niki E (2014) Biomarkers of lipid peroxidation in clinical material. Biochim Biophys Acta 1840:809–817

Noctor G, Mhamdi A, Foyer CH (2016) Oxidative stress and antioxidative systems: recipes for successful data collection and interpretation. Plant Cell Environ 39:1140–1160

Oliveira MT, Matzek V, Medeiros CD, Rivas R, Falcao HM, Santos MG (2014) Stress tolerance and ecophysiological ability of an invader and a native species in a seasonally dry tropical forest. PLoS One 9:e105514

Pintó-Marijuan M, Munné-Bosch S (2013) Ecophysiology of invasive plants: osmotic adjustment and antioxidants. Trends Plant Sci 18:660–666

Pintó-Marijuan M, Munné-Bosch S (2014) Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: advantages and limitations. J Exp Bot 65:3845–3857

Pintó-Marijuan M, Cotado A, Fleta-Soriano E, Munné-Bosch S (2017) Drought stress memory in the photosynthetic mechanisms of an invasive CAM species, *Aptenia cordifolia*. Photosynth Res 131:241–253

Pospísil P, Yamamoto Y (2017) Damage to photosystem II by lipid peroxidation products. Biochim Biophys Acta 1861:457–466

Qaderi M, Reid D (2008) Combined effects of temperature and carbon dioxide on plant growth and subsequent seed germinability of *Silene noctiflora*. Int J Plant Sci 169:1200–1209

Qaderi MM, Yeung EC, Reid DM (2008) Growth and physiological responses of an invasive alien species, *Silene noctiflora*, during two developmental stages to four levels of ultraviolet-B radiation. Ecoscience 15:150–159

Queval G, Noctor G (2007) A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: application to redox profiling during Arabidopsis rosette development. Anal Biochem 363:58–69

Queval G, Hager J, Gakière B, Noctor G (2008) Why are literature data for H_2O_2 contents so variable? A discussion of potential difficulties in the quantitative assay of leaf extracts. J Exp Bot 59:135–146

Quinet M, Descamps C, Coster Q, Lutts S, Jacquemart A-L (2015) Tolerance to water stress and shade in the invasive *Impatiens parviflora*. Int J Plant Sci 176:848–858

Ramel F, Birtic S, Cuiné S, Triantaphylidès C, Ravanat J-L, Havaux M (2012) Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiol 158:1267–1278

Redondo-Gómez S, Andrades-Moreno L, Mateos-Naranjo E, Parra R, Valera-Burgos J, Aroca R (2011) Synergic effect of salinity and zinc stress on growth and photosynthetic responses of the cordgrass, *Spartina densiflora*. J Exp Bot 62:5521–5530

Richards CL, Walls RL, Bailey JP, Parameswaran R, George T, Pigliucci M (2008) Plasticity in salt tolerance traits allows for invasion of novel habitat by Japanese knotweed s l (*Fallopian japonica* and *F. bohemica*, Polygonaceae). Am J Bot 95:931–942

Richardson AD, Duigan SP, Berlyn GP, Richardson AD (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytol 1:185–194

Roiloa SR, Retuerto R (2016) Effects of fragmentation and seawater submergence on photochemical efficiency and growth in the clonal invader *Carpobrotus edulis*. Flora 225:45–51

Roiloa SR, Rodrı S, Rube HF (2013) Developmentally-programmed division of labour in the clonal invader *Carpobrotus edulis*. Biol Invasions 9:1895–1905

Roiloa S, Rodiguez-Echeverria S, Lopez-Otero A, Retuerto R, Freitas H (2014) Adaptative plasticity to heterogeneous environments increases capacity for division of labor in the clonal invader *Carpobrotus edulis* (Aizoaceae). Am J Bot 101:1301–1308

Roiloa SR, Retuerto R, Campoy JG, Novoa A, Barreiro R (2016) division of labor brings greater benefits to clones of *Carpobrotus edulis* in the non-native range: evidence for rapid adaptive evolution. Front Plant Sci 7:1–13

Rotini A, Mejia AY, Costa R, Migliore L, Winters G (2017) Ecophysiological plasticity and bacteriome shift in the seagrass *Halophila stipulacea* along a depth gradient in the northern red sea. Front Plant Sci 7:1–12

Schneider C (2009) An update on products and mechanisms of lipid peroxidation. Mol Nutr Food Res 53:315–321

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012:1–26

Siegelman HW, Hendricks SB (1958) Photocontrol of alcohol, aldehyde, and anthocyanin production in apple skin. Plant Physiol 6:409–413

Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. Trends Ecol Evol 28:58–66

Song L, Chow WS, Sun L, Li C, Peng C (2010) Acclimation of photosystem II to high temperature in two *Wedelia* species from different geographical origins: implications for biological invasions upon global warming. J Exp Bot 61:4087–4096

Souza-Alonso P, González L (2017) Don't leave me behind: viability of vegetative propagules of the clonal invasive *Carpobrotus edulis* and implications for plant management. Biol Invasions 19:2171–2183

Spencer DF, Tan W, Liow P-S, Ksander GG, Whitehand LC (2008) Evaluation of a late summer imazapyr treatment for managing giant reed (*Arundo donax*). J Aquat Plant Manag 47:40–43

Takahashi S, Badger MR (2011) Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci 16:53–60

Takahashi S, Milward SE, Yamori W, Evans JR, Hillier W, Badger MR (2010) The solar action spectrum of photosystem. Plant Physiol 153:988–993

Thayer SS, Björkman O (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynth Res 23:331–343

Varone L, Catoni R, Bonito A, Gini E, Gratani L (2017) Photochemical performance of *Carpobrotus edulis* in response to various substrate salt concentrations. S Afr J Bot 111:258–266

Walters RG (2005) Towards an understanding of photosynthetic acclimation. J Exp Bot 56:435–447

Wang N, Yu FH, Li PX, He WM, Liu FH, Liu JM, Dong M (2008) Clonal integration affects growth, photosynthetic efficiency and biomass allocation, but not the competitive ability, of the alien invasive *Alternanthera philoxeroides* under severe stress. Ann Bot 101:671–678

Wang C, Liu J, Xiao H, Zhou J (2016) Differences in leaf functional traits between *Rhus typhina* and native species. Clean Air 44:1591–1597

Waring EF, Maricle BR (2012) Photosynthetic variation and carbon isotope discrimination in invasive wetland grasses in response to flooding. Environ Exp Bot 77:77–86

Weber D, Davies MJ, Grune T (2015) Determination of protein carbonyls in plasma, cell extracts, tissue homogenates, isolated proteins: focus on sample preparation and derivatization conditions. Redox Biol 5:367–380

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Curr Opin Plant Biol 5:218–223

Wu Y, Liu C, Li P, Wang J, Xing D, Wang B (2009) Photosynthetic characteristics involved in adaptability to Karst soil and alien invasion of paper mulberry (*Broussonetia papyrifera* (L.) Vent.) in comparison with mulberry (*Morus alba* L.). Photosynthetica 47:155–160

Yang L, Liu N, Ren H, Wang J (2009) Facilitation by two exotic Acacia: *Acacia auriculiformis* and *Acacia mangium* as nurse plants in South China. For Ecol Manag 257:1786–1793

Young AJ, Phillip D, Savill J (1997) Carotenoids in higer plant photosynthesis. In: Pessarakli M (ed) Handbook of photosynthesis, 2nd edn. Marcel Dekker, Tucson, p 1027

Yu HW, Yang JX, Gao Y, He WM (2016) Soil organic nitrogen endows invasive *Solidago canadensis* with greater advantages in low-phosphorus conditions. Ecosphere 7:1–10

Zhang LL, Wen DZ (2008) Photosynthesis, chlorophyll fluorescence, and antioxidant enzyme responses of invasive weed *Mikania micrantha* to *Bemisia tabaci* infestation. Photosynthetica 46:457–462

Zhang KM, Shen Y, Fang YM, Liu Y (2016) Changes in gametophyte physiology of *Pteris multifida* induced by the leaf leachate treatment of the invasive *Bidens pilosa*. Environ Sci Pollut R 23:3578–3585

Zheng YL, Feng YL, Lei YB, Liao ZY (2012) Comparisons of plastic responses to irradiance and physiological traits by invasive *Eupatorium adenophorum* and its native congeners. Plant Physiol 169:884–891

Zhou M, Diwu Z, Panchuk-Voloshina N, Haugland RP (1997) A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases. Anal Biochem 253:162–168

Zimmermann P, Zentgraf U (2005) The correlation between oxidative stress and senescence during plant development. Cell Mol Biol 10:515–534

Annex 2. Mort i plasticitat en espècies clonals en l'èxit invasor

Annex 2. Death and plasticity in clones influence invasion success.

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Forum

Death and Plasticity in Clones Influence Invasion Success

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Although invasion processes have been intensely studied, the mechanisms underlying the success of some invasive clonal species remain a mystery. Using the specific example of *Carpobrotus edulis*, we illustrate how invasion success can be facilitated by a unique spatiotemporal regulation of growth and senescence of plant parts.

Clonal Growth and Invasion Success

Although there is no single characteristic that can reliably predict the success of any particular species as an invader [1], clonal growth is considered an important trait for invasion success in several plant species [2]. Although this invasiveness may seem counterintuitive because species with clonal growth often have limited long-distance dispersion capacities, and because of their lack of variation-generating mechanisms of meiotic recombination and segregation, we propose here the 'grow and die strategy' as a process that facilitates invasion success in some clonal species. Using the specific example of *C. edulis*, a successful invader, we illustrate how invasion success can be facilitated by the development of a spatiotemporal continuum of growth and senescence of plant parts. We also discuss how integration and epigenetic changes across clonal generations may be advantageous for the growth of invasive clonal species.

Spatiotemporal Regulation of Growth and Senescence

Senescence may occur at either the level of the whole plant or the level of plant parts. In short-lived plants, such as annual and biannual (monocarpic) species, senescence occurs at the whole-plant level, promoting rapid nutrient remobilization to seeds and therefore favoring fast evolution. Short-lived plants are considered good invaders, since fast evolution will increase the rate of local adaptation in the invasive range [3]. In longer-lived clonal species, senescence of ramets (i.e., growth units deriving from a meristem) that have reproduced can also play a role in invasion success.

C. edulis, a highly succulent invader belonging to the Aizoaceae family and native to the Cape Region of South Africa, has been successful in invading some parts of California, Australia, and the Mediterranean basin, particularly in very hostile areas with low nutrient and water availability. In this species, once fruit maturation starts, ramets that have reproduced enter senescence. However, this process does not affect all of the plant; while ramets that have reproduced enter senescence, others can continue growing. By using clonal integration (physiological integration between ramets), this species increases its fitness [4,5] as well as its capacity for colonization of new habitats (Figure 1). Senescence of a significant part of the clone can result in autofacilitation through positive individual plant–soil feedback. A rapid turnover of plant biomass that results from rapid growth, death and regrowth creates an organic layer and transforms a hostile habitat into fertile soil for the next generation. A profound change in soil chemical properties results from the death of *C. edulis* ramets, leading to drastic changes in soil microbial activity and the deposition of a dense layer of organic matter in the soil [5,6]. This in turn promotes the renewal of soil layers generating an important source of rich nutrients and thus facilitating growth of the next generation into new areas. Important differences between soils have been shown before and after growth of *C. edulis* [5–7]. It should be considered, however, that the improvement of the soil might be different when whole ramets that have reproduced die compared with the addition of dead leaves from other ramets and roots, an aspect that requires further investigation. Furthermore, *C. edulis* has a strong allelopathic potential, thus negatively influencing the growth of other species [6].

Phenotypic Variation and Epigenetics

Clonal success may also depend on phenotypic variation that is mediated through epigenetic effects. Physiological integration can result in a mosaic individual increasing its potential invasiveness as ramets become better suited to local conditions. In a study on *Populus* in which replicate cuttings from genotypes were raised under different environmental conditions, it was shown that genetically identical ramets can show epigenetic and functional gene expression differences in different environments and that these differences can be transmitted to the next generation of ramets [8]. Transmission of epigenetic changes that are environment dependent will increase phenotypic plasticity, whereas epigenetic modifications that are heritable between generations of individuals will contribute to adaptive phenotypes through selective changes [9]. These epigenetic modifications can increase the adaptive match of a clonal phenotype to its environment and thus increase the fitness of the individual in that environment. Thus, a combination of sexual and asexual reproduction in invasive clonal species such as *C. edulis* may improve species fitness in a given environment. Furthermore, a recent comparative analysis between sexual and asexual species showed that asexual plants can change as often, and as fast, as sexual plants in response to novel environmental conditions encountered with range expansion [10]. Since asexual species show reduced epigenetic resetting due to their lack of meiosis, the epigenetic contribution to phenotypic plasticity and adaptation may be particularly important in clonal species [9]. Environmental effects on the phenotype from parents to clonal

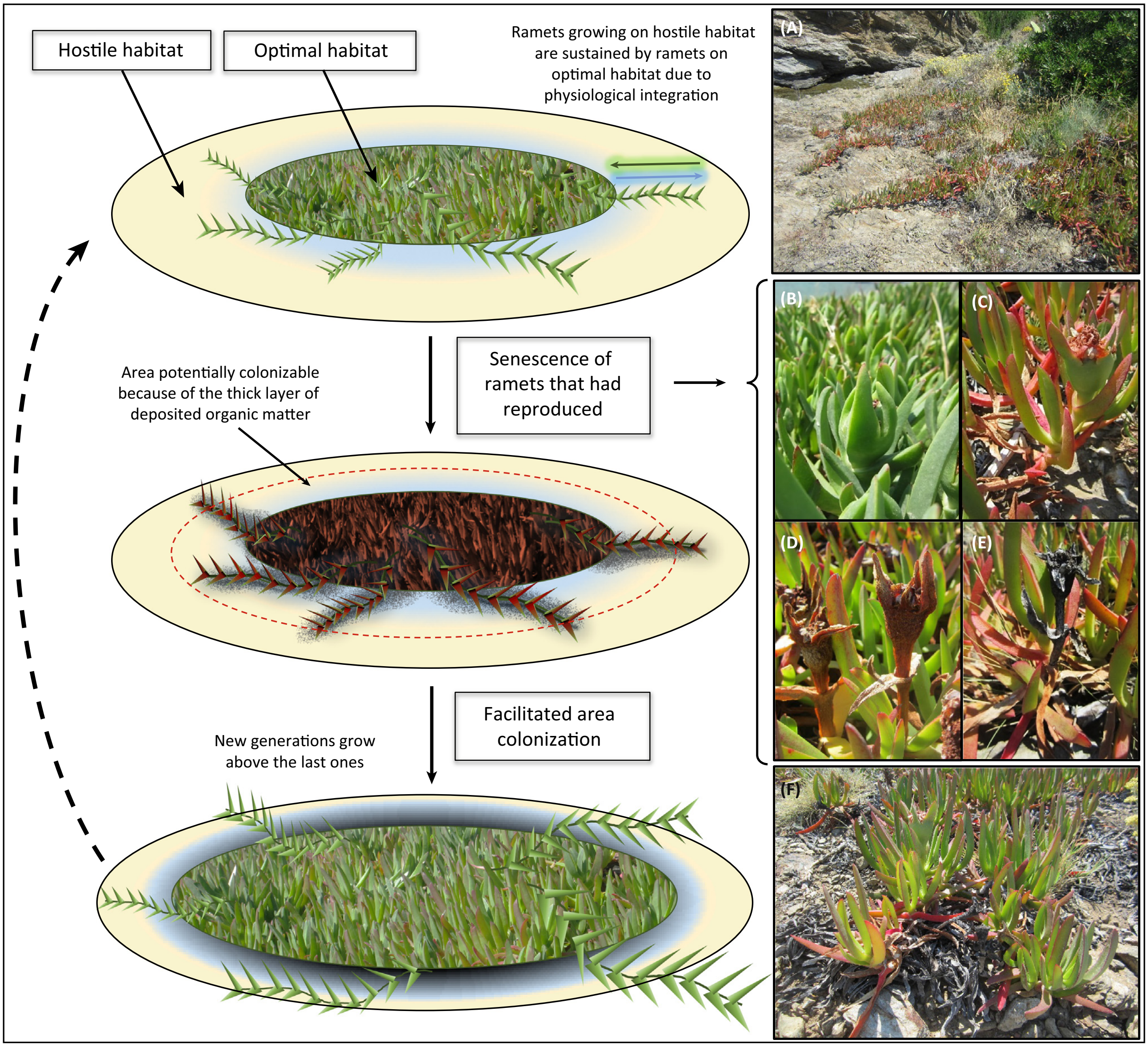

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Figure 1. 'Grow and Die Strategy' of the Invasive Plant *Carpobrotus edulis*. (**A**) Some ramets grow on and start colonizing a hostile habitat; (**B**–**E**) the sequence of senescence in some ramets that have reproduced; (**F**) some individuals growing above a thick layer of organic matter from the last generation. Photographs were taken in Far de s'Arenella, Catalonia, northeast Spain.

offspring have been demonstrated experimentally in a quantitative genetic study with *Eriphorum vaginatum*, an Arctic/Alpine sedge [11]. This species reproduces by seed following disturbance but primarily reproduces asexually through daughter tillers. Initially, tillers were grown in different levels of light and nutrients and then, over two more tiller divisions (generations), were grown at different nutrient levels. The results showed that the environment caused changes in the phenotype that were passed from parents to clonal offspring [11]. Similar to the influence of maternal effects in sexual organisms, the persistence of environmental and epigenetic effects in clonal plants may be considered clonal transgenerational, or clonal parental, effects.

The phenotype of a clonal species will therefore depend on its environment and the genotype of the meristem, which is responsible for producing both new

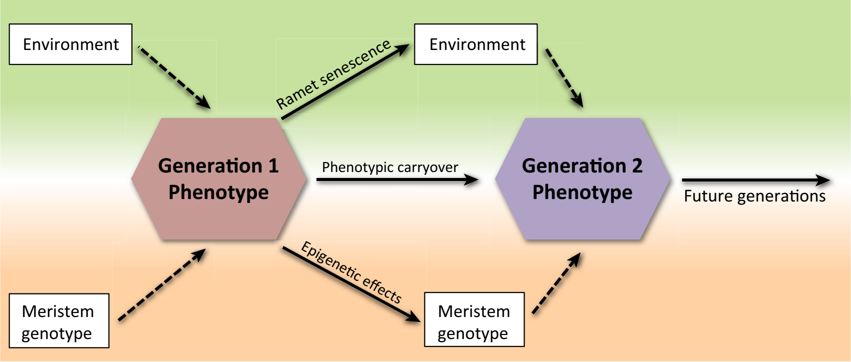

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Figure 2. Influences on the Clonal Phenotype across Generations of New Ramets. The phenotype in each generation is influenced by a clone's environment and meristem genotype (broken arrows). Unbroken arrows represent effects on the next generation's environment, phenotype, and genotype.

vegetative and reproductive growth (Figure 2). Also, as illustrated by *C. edulis*, the environment of successive clonal generations may also be influenced by ramet senescence. The combination of physiological integration and autofacilitation generates a colony growth pulse where ramet senescence seems to be a key to successful invasion as it modifies, and improves, the environment across generations. Epigenetic effects may in turn be a source of phenotypic plasticity and may explain some of the high levels of invasiveness in clonal species [9]. However, the significance of this variation and the persistence of the epigenetic inheritance across generations remains to be explored. According to the 'niche construction theory' [12], organisms receive from their ancestors not only genes but also a modified environment, and these two legacies determine the fitness of a population. This would be similar to the grow and die strategy, but in this case specifically operating in invasive plants with clonal growth.

Concluding Remarks

The grow and die strategy is used by some invasive clonal species to colonize new habitats. In *C. edulis*, a rapid turnover of plant biomass that results from rapid growth, death, and regrowth creates an organic layer and transforms a hostile habitat into fertile soil for the next generation. Senescence of plant parts, phenotypic variation, and epigenetic effects are all pathways through which the parental generation of a clone can influence the phenotype of its clonal offspring. However, the biological significance of these environmental and epigenetic effects in clonal plants needs to be studied in a broader range of species through the combined use of ecological, physiological, and genetic approaches to fully understand the importance of these effects to patterns of invasiveness. In the context of global environmental change and the challenges of managing the spread of non-native species, we need to understand how these effects may predict and contribute to invasion success and the impact of species on ecosystem-level processes.

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References

1. Van Kleunen, M. *et al.* (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecol. Lett.* 13, 235–245
2. Song, Y. *et al.* (2013) United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* 171, 317–327
3. Bossdorf, O. *et al.* (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144, 1–11
4. Roiloa, S.R. *et al.* (2013) Developmentally-programmed division of labour in the clonal invader *Carpobrotus edulis*. *Biol. Inv.* 15, 1895–1905
5. Conser, C. and Connor, E.F. (2009) Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. *Biol. Inv.* 11, 349–358
6. Novoa, A. and González, L. (2014) Impacts of *Carpobrotus edulis* (L.) N.E.Br. on the germination, establishment and survival of native plants: a clue for assessing its competitive strength. *PLoS ONE* 9, e107557
7. de la Peña, E. *et al.* (2010) Plant–soil feedback as a mechanism of invasion by *Carpobrotus edulis*. *Biol. Inv.* 12, 3637–3648
8. Raj, S. *et al.* (2011) Clone history shapes *Populus* drought responses. *Proc. Natl Acad. Sci. U.S.A.* 108, 12521–12526
9. Verhoeven, K.J.F. and Preite, V. (2013) Epigenetic variation in asexually reproducing organisms. *Evolution* 68, 644–655
10. Dalrymple, R.L. *et al.* (2015) Asexual plants change just as often and just as fast as do sexual plants when introduced to a new range. *Oikos* 124, 196–205
11. Schweagerle, K.E. *et al.* (2000) Quantitative genetics and the persistence of environmental effects in clonally propagated organisms. *Evolution* 54, 452–461
12. Laland, K. *et al.* (2016) An introduction to niche construction theory. *Evol. Ecol.* 30, 191–202

Annex 3. Un mètode ràpid i sensible per estimar la longevitat de les llavors d’una l’espècie invasora a través d’envelliment accelerat

Annex 3. A rapid and sensitive method to assess seed longevity through accelerated aging in an invasive plant species.

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A rapid and sensitive method to assess seed longevity through accelerated aging in an invasive plant species

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Abstract

Background: Seed longevity and vigor assessment is crucial for efficient ex situ biodiversity conservation in genebanks but may also have potential applications for the understanding of ecological processes and in situ biodiversity conservation. In fact, one of the factors determining the persistence of invasive species, a main threat to global biodiversity, is the generation of soil seed banks where seeds may remain viable for several years. Artificial seed aging tests using high temperatures and high relative humidity have been described for seed longevity estimation but have been mainly optimized for species with commercial interest. Thus, the aim of the study is to define a rapid and sensitive method to assess seed longevity and vigor through accelerated aging in the worldwide distributed invasive species *Carpobrotus edulis* to provide tools to biodiversity managers to evaluate invasive potential and develop effective post-eradication plans.

Results: Slow seed deterioration rate was obtained when *C. edulis* seeds were subjected to common accelerated aging temperatures (43–45 °C). This contrasts with the rapid viability decay between 24–72 h when seeds were subjected to temperatures superior to 55 °C, a strong inflection point for this species' thermosensitivity. Relative humidity also played a role in defining seed survival curves, but only at high temperatures, speeding up the deterioration process. The selected aging conditions, 55 °C at 87% relative humidity were tested over two *C. edulis* populations and three measures were proposed to parametrize the differential sigmoidal seed survival curves, defining the seed resistance to deterioration (L_5, aging time where 95% of seeds maintain their viability), medium longevity (L_{50}, 50% of seeds lose their viability) and lethal aging time (L_{95}, 95% of viability loss).

Conclusions: An accelerated aging test at 55 °C and 87% relative humidity constitutes a rapid and sensitive method that can be performed within a working week, allowing managers to easily test seed vigor and longevity. This test may contribute to assess invasive potential, design effective monitoring programs and soil seed bank eradication treatments.

Keywords: Aizoaceae, Deterioration, Germination, Management, Post-eradication, Seed survival curve, Soil seed bank, Solarization, Temperature, Thermotolerance

Background

Seed longevity is described as the period over which a seed remains viable and capable of germination [1]. The length of time during which seeds maintain their viability and vigor is a trait of interest not only for crop species within breeding programs and food security, but also for describing natural populations' dynamics and assess

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persistence potential [2–4]. Seed longevity is strongly variable, ranging from just a few months in soybean [5], to more than 2000 years in *Phoenix dactylifera* [6]. In species with orthodox seeds, viable seeds may enter dormancy, a temporal suppression of germination under favorable conditions, thanks to their ability to tolerate considerable desiccation [7]. The slow pace of seed viability loss in orthodox species hinders the study and characterization of species' seed longevity under natural conditions. Due to those difficulties, ex situ aging tests are used to estimate seed longevity and vigor [8]. Genetic factors have a key influence on seed persistence and because of this, the survival of seeds experiencing moisture and temperature stress in the laboratory is thought to be a good predictor of their potential persistence in the environment [1]. In fact, similar molecular events were found to accompany artificial and natural seed aging processes [9]. Long et al. [10] successfully predicted species seed persistence in the field with the species ranking of the days taken for seed viability to decline by 50% in an artificial aging test. A faster viability decay under artificial aging conditions is linked to a germination depression with time [11]. There are different proposed approaches to estimate and contrast seed longevity by generating an atmosphere that speeds up the aging process: accelerated aging and controlled deterioration. Controlled deterioration tests were first developed to identify low vigor seeds [12] and consist of subjecting to high temperatures (around 40–45 °C) prearranged high seed moisture content (from 18 to 24%) for 24 to 96 h [13]. In contrast, accelerated aging (AA) tests consist of exposing seeds to high temperatures around 40–45 °C over water, generating an atmosphere with high relative humidity (RH). The AA test was first developed by Delouche and Baskin [14] to estimate the longevity of seeds stored in a warehouse, as the decline in germination following AA is proportional to the initial physiological potential of the seed lot. Seed lots with high vigor should be able to withstand these stress conditions and will deteriorate at a slower rate than lots with poorer vigor [8]. The AA test is preferable for a rapid method as it does not require initial seed moisture control and equilibration, which may suppose extra time and offers similar results to the controlled deterioration test [13].

Standard AA conditions proposed for several crop seeds varies between 41 and 43 °C for 48 to 72 h [15]. However, even within a species, seed longevity in AA tests may depend on several factors such as moisture content, RH, oxygen partial pressure and temperature [16]. Higher temperatures and high RH are known to boost seed viability loss [11] but may have different effects in different species. Artificially aged seeds of rice lose their viability between 25 to 45 days at 40 °C and 80% of RH but just in 6 days at 45 °C and 100% RH, whereas *Arabidopsis thaliana* seeds lose their viability within 3 days at 37 °C at 83% RH [8]. Artificially aging protocols have been mainly designed for grain, vegetable, forage, and forestry crops [17] such as rice, wheat, soybean and tomato, leaving aside other species with ecological interest that could offer new insights into seed longevity regulation and over which an optimized AA test could be beneficial to assess ecological dynamics [2]. Artificial aging tests may be useful to test seed lot status and predict when the seeds may reach the viability threshold that may require further actions to conserve the species' germplasm [18], thus improving genebanks' management for ex situ germplasm conservation [19]. Non-crop species' germplasm conservation may be important for maintaining biodiversity's option value (i.e. the value of potential future benefits that are unknown today, such as finding new pharmacologically-beneficial substances), in spite of the difficulties of defining this concept [20–22].

Invasive species constitute one of the five major drives of global biodiversity loss [23] and eradication costs increase with invasion time specially for those invaders that are able to generate a permanent soil seed bank [24]. *Carpobrotus edulis* (L.) N. E. Br. (Aizoaceae) is a mat-forming trailing succulent perennial that has been introduced into all continents, mostly growing in the Mediterranean regions [25]. This species is native from South Africa where it is used in traditional medicine for its several pharmacological applications for the treatment of tuberculosis and other respiratory infections, toothache and earache, facial eczema, wounds, burns, hypertension, and diabetes mellitus, due to the antimicrobial, antiproliferative, and antioxidant properties of *C. edulis* leaf extracts [26]. Despite its pharmacological potential, in the introduced range, this species impacts native communities by decreasing biodiversity and altering nutrient cycling dynamics [27, 28]. The production of allelopathic compounds contribute to the drastic modification of this species surroundings inhibiting native species germination [29]. One of the factors determining this invasive species' persistence is the intense flowering and high amount of small hard-coated reniform orthodox seeds produced, which generates a permanent soil seed bank [30]. Invasive species eradication costs are extremely high in advanced invasion stages, especially because local eradication is not possible as long as seeds remain viable in the soil. In weed eradication and control programs it is critical to know the characteristics of the soil seed bank for the implementation of successful control measures and monitoring [31]. Some ex situ techniques have been proposed to predict the persistence of seeds and to enable policy makers to make faster and better-informed decisions in weed-management programs [32]. Assessing seed longevity in invasive plants becomes crucial for management policies when prioritizing zones or determining budget allocation in post-eradication plans. Considering *C. edulis* wide distribution, its ecological impacts and the fact that it is able to generate a permanent soil seed bank that increase eradication costs, we aimed to describe a rapid AA test for the seeds of this invasive species, evaluating the role of different parameters to estimate seed vigor in natural populations. We tested over different natural populations the optimal conditions that generate a progressive viability loss following the sigmoidal response described for seed aging within a short time period (less than 10 days), maximizing feasibility with low economic costs. This test may be useful for management programs to assess invasive potential, define eradication strategies and promote effective genebank management in non-crop species.

Results

The different experimental conditions tested lead to the understanding of the importance of several factors determining artificial aging seed survival curves in *C. edulis*. All artificial aging tested conditions lead to a loss in the initial seed viability, but its timing was strongly influenced by the induced conditions. When it comes to keeping the aging environmental stable, some treatments were found more feasible than others, which helped to decide the best artificial aging environment for *C. edulis* seeds in terms of speed, feasibility and simplicity. The final aging protocol (resumed in Fig. 1) was defined at 55 °C and 87% RH and revealed differential seed survival curves between the two tested natural populations.

Temperature

Temperature strongly influenced seed survival curves and their medium longevity (Fig. 2). The lowest tested temperature (43 °C) led to a constant and unhurried loss of seed viability of only a 20% loss in 18 days (Fig. 2a). Similar aging pace was observed at 45 and 50 °C but an

Fig. 1 Eight-step accelerated aging test optimized for *C. edulis*. Eight steps describing the accelerated aging protocol from seed collection (step 1) to classification of stained *C. edulis* embryos after tetrazolium (TZ) incubation for viability testing according to the staining intensity and distribution (step 8)

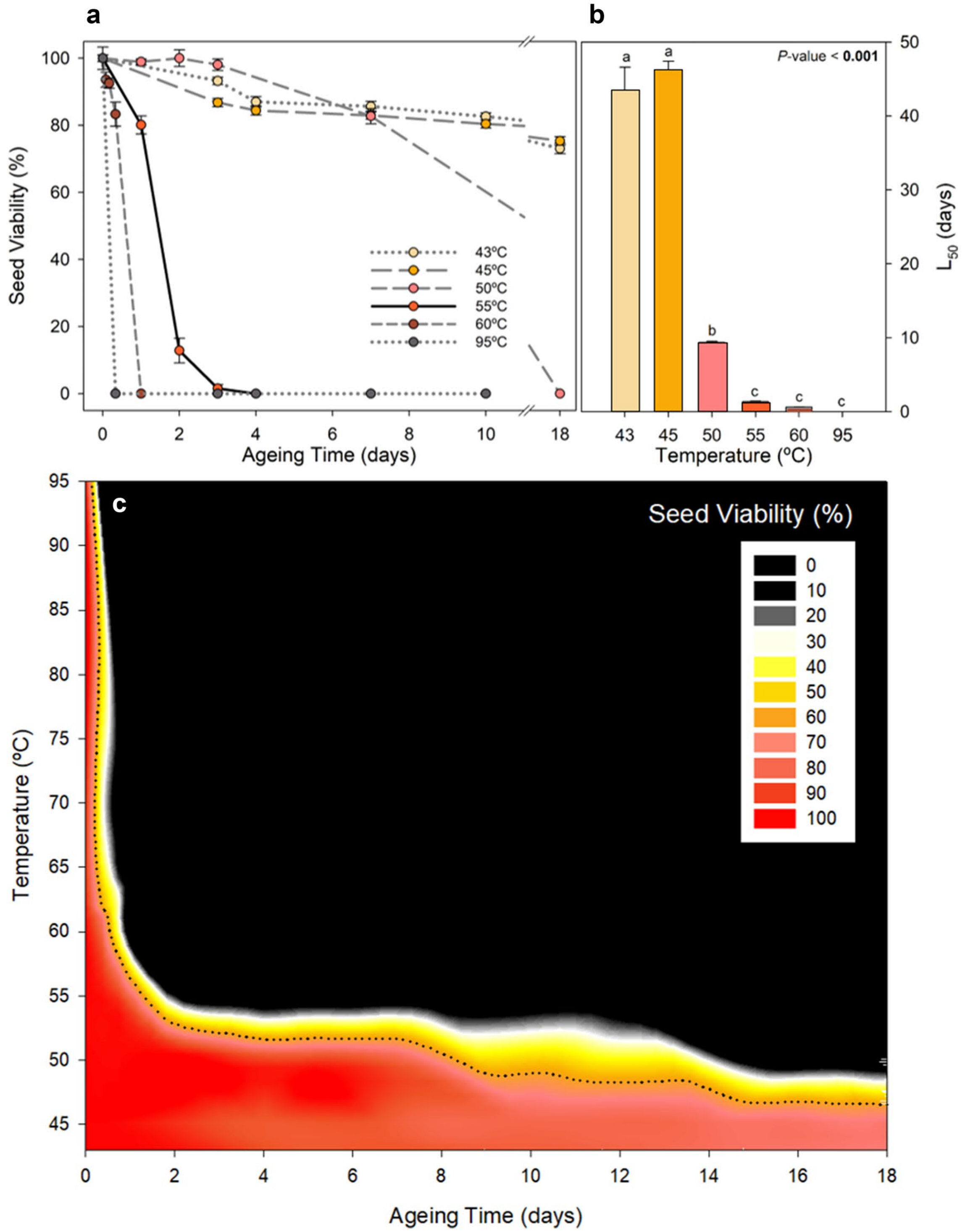
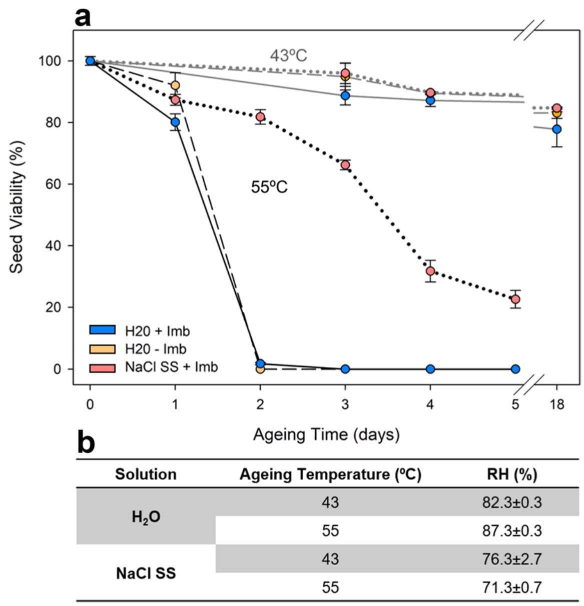


Fig. 2 Influence of temperature and aging time in artificially aged seeds of *C. edulis.* **a** Viability loss of *C. edulis* seeds at the different tested temperatures and 87% RH. Data is represented as Mean ± SE ($n = 6$). **b** L_{50} (loss of 50% viability) at the different tested temperatures. Different letters indicate statistically significant differences (P-value < 0.05). **c** Contour plot of seed viability considering temperature and aging days. Dotted line represents the L_{50}, 50% viability loss

abrupt change was observed at 55 °C, leading to the typical sigmoidal seed survival curve (Fig. 2a). Higher temperatures induced 100% viability loss between 3 and 24 h under 60 °C and less than 3 h under 95 °C (Fig. 2a). Those temperatures led to the apparition of burned embryos with brownish coloration. The L_{50}, the time required to reach a 50% viability loss decreased sharply with increasing temperatures, from around 45 days under 43-45 °C to just a few hours at 60 °C (Fig. 2b). Temperatures above 55 °C may offer artificial aging intervals within a week, as L_{50} was found between 2 and 3 days of aging treatment. Seed viability did not decrease linearly with temperature and aging time, instead, a strong inflection point was observed around 55 °C when contrasting aging time, temperature and seed viability (Fig. 2c).



Solution	Ageing Temperature (ºC)	RH (%)
H_2O	43	82.3±0.3
	55	87.3±0.3
NaCl SS	43	76.3±2.7
	55	71.3±0.7

Fig. 3 Relative humidity in interaction with temperature and imbibition effect in aging seeds of *C. edulis*. **b** Temperature and RH conditions in the aging recipient for the different tested conditions. NaCl SS: NaCl saturated solution. Data is represented as Mean ± SE (n = 6). Temperature, Aging time and Relative humidity effects were strongly significant (*P*-value < 0.01), whereas imbibition had non-significant effects (*P*-value = 0.328)

Relative humidity and seed imbibition

The amount of water vapor in the aging atmosphere determined differences in seed survival curves. The superior RH obtained in the 82-87% RH experiment (using distilled water) determined an accelerated viability loss in contrast with the 71–76% RH experiment (using a saturated salt solution, NaCl) (Fig. 3). This difference was observed under 55 °C but not at 43 °C, where no differences in the seed survival curve were observed (Fig. 3). When contrasting treatments at 55 °C, high RH (87%) lead to a complete viability loss after two aging days, whereas a lower humidity (71%) triggered a progressive viability loss that leave 20% viable seeds after five aging days (Fig. 3). Apart from a different seed survival curve, the imbibed seeds in low RH conditions (71%) at 55 °C were found with a hardened seed coat at all aging times, hindering seed piercing for viability analysis.

Seed imbition did not exert any significant effect on seed survival curve at any aging time when compared with non-disinfected dry seeds (Fig. 3a). However, clear practical benefits were found when placing seeds on the aging container with water using a Pasteur pipet, instead of placing them one by one in dry with tweezers. In spite of the fact that imbibed seeds and non-disinfected dry seeds showed no significant differences within the viability loss interval (0–120 h), the lack of seed disinfection promoted fungus attacks from the 7th aging day in some replicates, which contributed to discard this treatment.

Viability and germination's potential loss

The seed survival curves in terms of viability and germination tested in the two different populations of *C. edulis* triggered two differentiated responses. Initial seed moisture content was not significantly different (*P*-value > 0.05) between the two populations: with 13.54 ± 0.82% and 14.22 ± 0.64% in population 1 and population 2 respectively. Population 1 followed a sigmoidal pattern with an abrupt fall within the 40 and 80 aging hours range, whereas population 2 showed a slow viability loss without a resistance phase (Fig. 4). Initial viability was found very high (around 70–90%), whereas initial germination percentage fell to 20% in Population 2. The viability loss was accompanied by an increase in the proportion of dying seeds in both populations (patchy and patchy-weakly embryos, Fig. 4b). Those dying seeds were unable to germinate as shown by the null germination rates found after 72 aging hours (Fig. 4c). Germination time was initially similar between the two populations and maintained at 40 days until 24 aging hours. Whereas population 1 maintained mean germination time after 48 aging hours, population 2 showed a significant decay at this time. Similarly, a strong decrease in the mean germination rate was observed past the 48 aging hours (Fig. 4).

Seed longevity estimation

A non-linear response was found for viability loss with aging time (Fig. 4a). The best fit for the sigmoidal curve of the seed survival was observed using Weibull model adjustment. This model allowed the estimation of different parameters (Fig. 4a). The best fitted viability loss for population 1 was a Weibull type 2 with three parameters:

$$V = 83.06\left(1 - e^{-e^{-9.3(\log(x) - \log(52.33))}}\right)$$

Whereas the best-fitted viability loss for population 2 was a Weibull type 1 with three parameters:

$$V = 72.64e^{\left(-e^{1.65(\log(x) - \log(40.61))}\right)}$$

where V stands for Viability (%), and x for aging time in hours. To characterize seed survival curve we propose the use of three different parameters that reflect: aging resistance (L_5), mean longevity (L_{50}) and lethal aging time (L_{95}) under aging conditions, that define the number of hours to achieve 5, 50 and 95% of viability loss respectively. Those parameters characterize the two differentiated seed survival curves obtained for the two natural populations. The lack of seed resistance to aging conditions of population 2 was reflected in the small L_5 (6.73 ± 1.25 h) in comparison with population 1 (46.51 ± 0.80 h) (Fig. 4). Moreover, different L_{50} reflected the medium seed longevity that also differed between populations and was found between 32 and 54 aging hours. Lethal aging time (L_{95}) was found past the 72-79 aging hours in both populations.

Discussion

Seed resistance to temperature and relative humidity

Temperature played the most important role in reducing seed longevity in *C. edulis*. Increasing temperatures are described to reduce the accelerated aging time, with seeds reaching the 0% viability in a shorter time [11]. Substantial decrease in the activity of antioxidants in response to increased accumulation of reactive oxygen species is associated with accelerated aging [33–36]. If not counterbalanced, reactive oxygen species lead to the accumulation of damaged molecules such as lipid hydroperoxides and protein carbonylation that increase strongly in deteriorating seeds and may ultimately lead to seed death [37, 38]. Seeds may respond to high temperatures with seed death (non-tolerant seeds), non-altered viability (tolerant seeds) or trigger increased germination (i.e. pyrophile plants) [39, 40]. Seed survival curves may vary depending on specific temperature and exposure time [41]. In the case of *C. edulis*, 5 °C increase from 45 to 50 °C were enough to reduce 4 times the L_{50}, suggesting a strong sensitivity to temperature. Moreover, *C. edulis* seeds did not tolerate 3 h exposure at 95 °C as other Aizoaceaceae may do according to the study developed

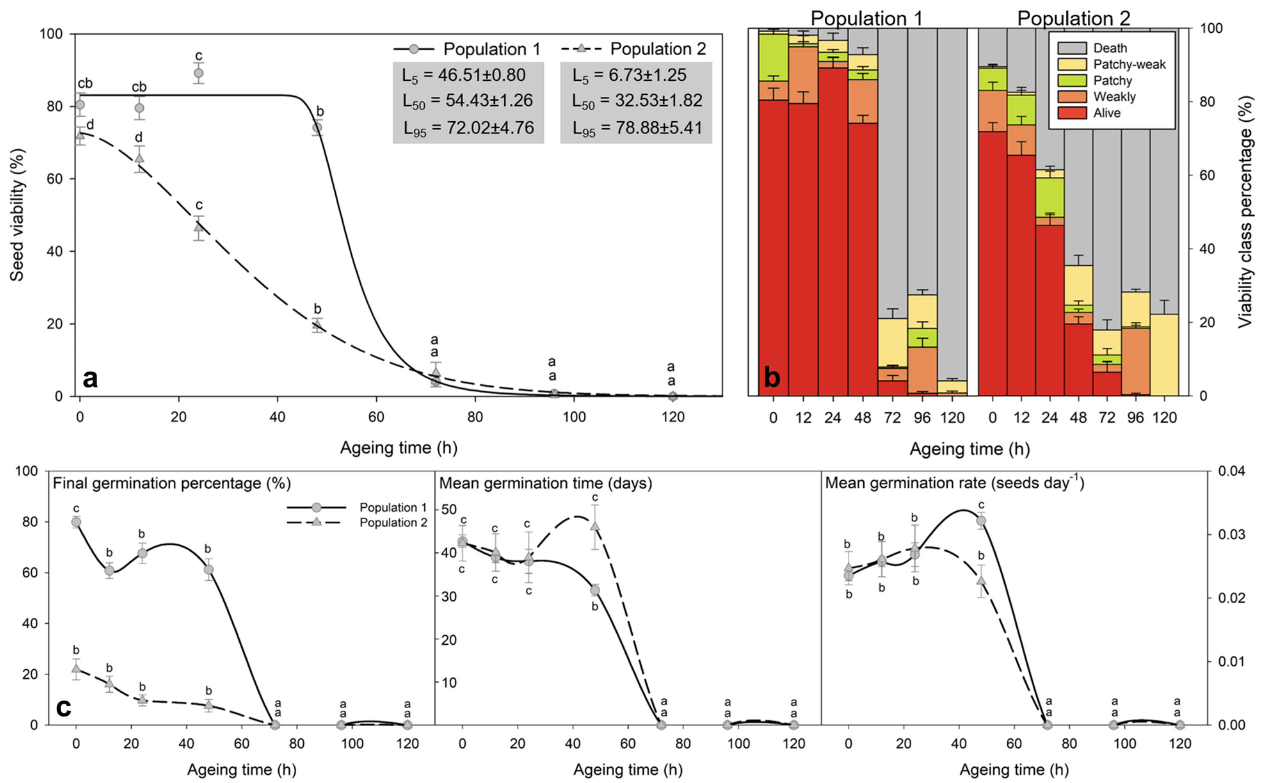


Fig. 4 Viability and germination loss with aging time at 55 °C and 87% RH of *C. edulis* seeds from two natural populations. **a** Viability loss and obtained values for L_5, L_{50}, L_{95} for each population. Black lines correspond to best fitted Weibull model for each seed lot (n = 35). **b** Percentage of embryo classes during aging in *C. edulis* seeds considering the two populations. **c** Germination parameters variation with aging time. Different letters in **a** and **c** indicate statistically significant differences (*P*-value < 0.05) within a population. Data is represented as Mean ± SE (n = 5)

by Daws et al. [42], where at least 20% seed survival was observed in 26 Aizoaceae species at 103 °C for 17 h. Seeds of *Welwitschia mirabilis* from the Namib Desert have been reported to tolerate 80 °C for 48 h [43]. However, those studies were orientated to evaluate seeds thermotolerance and low RH was used. High RH (up to 75–80%) is recommended for testing seed longevity [8]. Differences in RH generate differential seed survival curves in *C. edulis* seeds under sensitive temperatures, with higher RH (87%) promoting a faster seed aging in comparison with lower RH (73%). This result coincides with those obtained in *Jatropha curcas* L. seeds, which showed a faster deterioration in AA tests over water in contrast with using a saturated salt solution [44]. Similar results were obtained with *Hirschfeldia incana* when comparing humid (75%) and dry (33% RH) storage at 35 °C, where humid storage decreased almost 100 times seed longevity [45]. In fact, resultant products of non-enzymatic modifications of protein structures through glycation found in accelerated aged soybean seeds were formed most rapidly in seeds at high humidity [46]. The inactivation of proteins would depress the metabolic capacity and reduce the ability of biological systems to repair cellular damage occurring during storage [47].

When contrasting aging tolerance of *C. edulis* seeds with conventional artificial aging protocols for commercial seeds, *C. edulis* appears to have an increased tolerance. Considering that the general temperature for artificial aging is between 41 and 43 °C for 48 to 72 h [8, 15], *C. edulis* seeds may be considered resistant, as under those temperatures, viability was maintained above 80% at least for 10 days. Moreover, 80% of *C. edulis* seeds survived after a heat shock of 3 h at 60 °C, whereas imbibed seeds from other species may not even survive a 30-min shock at this temperature [48]. *Ex-situ* seed longevity was found to be correlated with drought exposure during the post-zygotic phase in dry and warm environments using 16 closely-related populations of the genus *Silene* [49]. Post-zygotic *C. edulis* phase occurs during summer, coinciding with low water availability and the highest yearly temperatures, [50] which may contribute to explain increased ex situ seed longevity in comparison with other commercial species subjected to similar AA tests.

Optimal conditions for artificial aging and measurement of seed longevity

The optimal conditions for AA test in *C. edulis* were selected considering speediness, simplicity and sensitivity. The chosen conditions for artificial seed aging were 55 °C over water (87% RH) using imbibed *C. edulis* seeds. The chosen temperature and relative humidity offer the possibility to test seed longevity within a week, as longevity is lost in 3–5 days. Moreover, 55 °C constitutes the inflection point in the viability loss-aging time curve suggesting high sensitivity to changes in seed longevity at this temperature. Besides the advantageous temporal scale of using 55 °C and a water solution, it also requires less preparation than using a saturated salt solution and avoids the risk of salt crystallization over seeds. Finally, seed imbibition and disinfection did not lead to any significant difference and offered substantial practical benefits as seed softening, disinfection facilities and seed disposal in the aging containers pipetting seeds instead of nipping them one by one.

Seed survival curve typically shows a lag phase before seed viability starts to decline rapidly [8]. This was the observed response for *C. edulis* seeds to selected artificial aging conditions, in both the analysis of seed viability (which took two working days in a 3 days' time interval) and seed germination (which took 70 days of germination control every 3 days) coinciding in duration of the lag phase and survival loss in the two *C. edulis* populations. Viability measures may be equally accurate and may be less time-consuming than germination measurements, as also exposed in Xu et al. [51]. Although both populations showed similar seed viability (around 70-80%), population 2 showed a 25% germination, revealing that 45% of viable seeds did not germinate, which may correspond to dormant seeds. Viability test is also preferable in comparison to germination test as it reflects seed lot viability regardless of their dormant state [52]. The characterization of the tipping point in the modeled sigmoidal curve of viability loss before the sharp decline is essential, corresponding to exhaustion after the resistance phase in seed stress responses, characterized by a failure of protection and repair and critical cell death [53]. To characterize the duration of the resistance phase, L_5 (the aging time when 95% of the initial viable seeds remain viable) is here proposed. Company et al. [4] have proposed P_{20} (the aging time to decrease a 20% seed survival) to characterize the first survival loss in the curve but this measure may fall very close to the L_{50} and will not provide new information. The aging time that causes a 50% decrease in seed survival has been largely accepted as a measure of seed longevity [8]. Besides this measurement, the point where seeds no longer germinate (P_0) was proposed by Dowsett et al. [2] to be a better measurement of seed longevity due to the rapid decline in germination. In this way, Company et al. [4] also proposed the use of P_{80} (80% of seed mortality). We suggest that L_{95} (the point where only 5% viability is observed) may be a better indication of the lethal seed aging time when estimating parameters from a fitted curve. The triplet of parameters: L_5, L_{50} and L_{95} may provide information on sensitivity, medium aging time, and maximum resistance of a seed lot.

Artificial aging to assess invasive potential

Dowsett et al. [2] suggested that artificial aging tests may have a valuable role in understanding the biology of weed species. Besides understanding their biology, artificial aging tests may contribute to design effective management policies and eradication strategies. Recently, Company et al. [4] evaluated the potential competitive displacement of the invasive species *Cortaderia selloana* over the native *Saccharum ravennae* using seed germination and viability tests under AA conditions and revealed that no long periods of subsequent monitoring are needed after eradication of *C. selloana*, as this species presented low seed longevity. The two *C. edulis* populations where the AA test was tested showed different seed longevities towards an increased seed vigor in population 1, that may require increased eradication efforts. Accelerated aging tests on *C. edulis* may allow rapid assessment by managers and policy makers of seed's vigor at different zones and may contribute to design effective monitoring programs taking into account seed longevity estimations with appropriated budget allocation. Moreover, post-eradication soil seed bank vigor monitoring may contribute to define re-invasion risks and estimate weed eradication programs [32].

The artificial aging test optimized contributes to the understanding of invasive seed thermosensitivity that may be used to design soil seed bank eradication treatments. In this way, soil solarization is a non-chemical environmentally-friendly agricultural method for soil disinfestation. Using transparent polyethylene sheets, the temperature of the soil at a depth of 0–20 cm usually reaches 40–60 °C, leading to the eradication of pathogens, arthropod pests, and weeds. This soil treatment has been already tested to manage invasive seed banks and lead to significantly reduced viability of buried *Acacia* seeds, including the invasive species *A. saligna*, *A. murrayana* and *A. sclerosperma*, by exposing seeds to lethal hydrothermal conditions, constituting an effective method to reduce the persistent invasive seed bank of those species [54–56]. Considering the *C. edulis* seeds' thermic sensitivity described here and the fact that a 77.6% of this species' soil seed bank is mainly found in the species' litter and the first 0-5 soil cm [30], soil solarization may constitute a promising tool for invaded soil seed bank eradication. Just 24 h at 60 °C and 87% RH may be enough to obtain a complete viability loss in *C. edulis* seeds. Midday soil surface temperatures in the range 50–80 °C have been reported in environments ranging from tropical forests to deserts [57, 58], and 60 °C may be easily obtained under polyethylene sheets with air temperature reaches 30 °C [56]. However, further research is needed to determine the effectivity of a soil solarization treatment under natural soil moisture conditions and the effect of thermal cycles versus the sustained temperatures explored here.

Conclusions

The inflection point found with temperature-aging time analysis led to the definition of a rapid and sensitive method for *C. edulis* seeds artificial aging at 55 °C under 87% RH, where viability is lost within a working week, allowing managers to easily test seed vigor and longevity to assess invasive potential and contribute to design effective monitoring programs taking into account seed longevity estimations with appropriated budget allocation at different invaded areas. *C. edulis* seed thermosensitivity has been addressed suggesting that soil solarization treatments that mimic the described conditions may be effective for this species' soil seed bank eradication. The optimization of artificial aging tests in non-crop species may provide new insights into seed longevity regulation and develop efficient management practices for ex situ germplasm conservation.

Methods

Plant material

A first lot of *C. edulis* seeds was selected to test the aging conditions from a natural population growing in Premià de Mar (Spain, 41° 29′ 24.2″ N 2° 21′ 49.3″ E). Once aging conditions were set, seeds from two natural populations of *C. edulis* were selected because of their large number of individuals and their large seed availability (population 1: 41° 28′ 25.5″ N 2° 17′ 54.7″ E; population 2: 41° 28′ 30.3″ N 2° 18′ 04.6″ E). These populations were used to test the accelerated aging test. From each population, within the period of natural seed rain, 600 fruits were collected to ensure representativeness. Fruits were opened and all the obtained seeds were pooled (more than 10.000 seeds per population). Seeds were kept at darkness and room temperature until analysis (3–4 weeks). Prior to start the accelerated aging tests, seed moisture content (%) was measured for both populations as: FW − DW*100/DW, where FW stands for 50 seeds fresh weight and DW for those 50 seeds dry weight (obtained after 72 h at 70 °C) with five replicates. In the two populations, accelerated aging through viability tests and germination was tested as described in the following sections.

Accelerated aging test

Different containers were tested regarding their surface/volume ratios to maximize seed disposition and effectivity and preservation of the aging conditions. It was concluded that a commercial polypropylene hermetic container of 1.1L capacity with a 20 × 5 cm surface (PlasticForte, Spain) was ideal to generate a controlled atmosphere that maintains the desired RH and hold the seeds at a determined point with 70% of the capacity filled with aging solution (Fig. 1). There was an exception at 95 °C where due to the temperature, the experiment was performed using glass jars. Five replicates per population, including fifty seeds per replication were used.

The preparation of the aging containers was performed as follows: first, containers and sized meshes were disinfected with 70% ethanol and filled with 700 ml of aging solutions: water or NaCl saturated solution (See relative humidity experiment below). Mesh was fixed using masking tape. Seeds were submerged in 0.1% bleach for 30 s and 10% ethanol for 30 s more and washed multiple times with distilled water. Containers were only opened when the aging treatment ended at the chosen time: from 3 to 18 days. At that moment, seeds were prepared for the viability test as described in the succeeding sections. Zero aging hours corresponds to viability (through tetrazolium viability test) or germination assessment on non-artificially aged seeds for viability and germination tests respectively.

Temperature experiment

The role of aging temperature, RH and seed imbibition and disinfection on the seed survival curve in terms of seed viability was tested. The general aging procedures suggest the use of 41–43 °C in a high humidity environment for a specified duration (48–72 h) [8, 15]. However, seeds from some Aizoaceae species are known to resist even higher temperatures for a short period of time [42]. Considering this, aging was tested at the following spectrum temperatures: 43, 45, 50, 55, 60, 95 °C using a laboratory oven under high humidity conditions using distilled water aging solution inside the containers that generated an atmosphere of 87% RH.

Relative humidity and seed imbibition experiment

To evaluate the role of RH inside the aging container, distilled water and a saturated commercial NaCl solution (40 g/100 ml H_2O) were used. Saturated salt solutions are often used for controlling environmental variables as they generate a defined RH environment, inferior to a water-saturated atmosphere [44]. Relative humidity and temperature inside the aging container were tested by introducing a data logger sensor (EasyLog, Lascar Electronics) and a wireless digital thermometer and hygrometer (WA10-Kira EU, Oria) after different time intervals (24, 48, 72 and 96 h inside aging containers).

Seed imbibition is recommended in tetrazolium viability tests (see seed viability section) because it softens the seed coat and activate enzymatic systems [52]. In order to standardize conditions for initial viability measurement and accelerated aging conditions, imbibition was also proposed before accelerated aging. Seed imbibition was performed with distilled water during 24 h (for its compatibility with the normal laboratory routine) in the dark and at room temperature. Unlike imbibed seeds, dry seeds were not disinfected to maintain the dry conditions and compare the seeds dry original state and the imbibed and disinfected seeds. Using a Pasteur pipette for imbibed and tweezers for dry seeds, seeds were distributed into the 10 cm^2 filter paper that was placed on the containers' mesh together with other replicates.

Seed viability

To asses seed viability at the different aging times, the tetrazolium (triphenyl tetrazolium chloride) test was used [15, 52, 59]. Seeds were delicately pierced under a binocular magnifying glass to ensure tetrazolium penetration. No staining was observed in non-pierced seeds. Five replications per population, including fifty seeds per replication were disposed in 2 ml capacity eppendorfs with 0.5 ml of 0.1% tetrazolium (Sigma-Aldrich, Steinheim, Germany) and was incubated for 48 h at 40 °C before viability assessment (Fig. 1). Tetrazolium incubation conditions were chosen following the recommendations from standard procedures [15, 52, 59] that suggest higher temperatures (within the defined accuracy range 20–40 °C) to speed up the staining process. Tetrazolium staining time was evaluated within the interval described for some species from 2 to 48 h [52, 59] and staining time inferior to 48 h revealed incomplete stained embryos. After two days, tetrazolium was withdrawn using a 1 ml pipette and 0.5 ml of distilled water was added to each eppendorf to facilitate further seeds displacement. Using a Pasteur pipette, seeds were spread in a petri dish under a binocular magnifying glass and seed viability was assessed using tweezers. A little squeeze was enough to force the embryo to escape the seed coat and determine its vigor. Six different categories were used to classify embryo status: viable (totally stained), weakly (pale stained), patchy (partially stained), patchy-weakly (partially pale stained), death (white embryo), and aborted (no embryo found) (Fig. 1). The sum of weakly, patchy and patchy-weakly seeds constitutes low vigor seeds without survival probabilities (dying). To speed and facilitate data collection, three smart phone free apps were used for voice recording during embryo viability classification (Voice recorder from Splend Apps), afterwards fast reproduction (Music Speed Changer from Single Minded Productions, LLC), and class counting (Thing counter from Karuma).

Seed germination

Seed germination was performed as described in Novoa et al. [60] and Podda et al. [61] with brief modifications on artificially aged seeds of populations 1 and 2 for the different aging times (0, 12, 24, 48, 72, 96 and 120 h). Different conditions of pH, temperature alternation and acid pre-treatment were tested, obtaining the best results with 12 h photoperiod with temperature alternation between 25 °C during the day and 10 °C at night. Germination was assessed every three days over wet Whatman paper (90 mm No. 1 filter) in petri dishes. Five replications per population, including fifty seeds per replication were used. Final germination percentage was estimated after curve saturation at 70 days. Mean germination time and mean germination rate were estimated using the *GerminaR* package in R 3.5.0.

Data analysis

Significant differences between aging time or temperature were tested using a one-way ANOVA, evaluating the effects of the fixed factor 'Aging time' for the different variables in Fig. 4 and the fixed factor 'Temperature' in Fig. 2b using Tukey test to contrast differences between factor levels in R 3.5.0. A four-way ANOVA was used to test the effects of the three fixed factors: 'Relative humidity', 'Temperature', 'Aging time' and 'Imbibition', for the times 0, 24, 48 72, 96 h, assuming that viability at 43 °C after 24 and 48 h was equal to 72 h in Fig. 3a. Normality and homoscedasticity were confirmed using the Shapiro–Wilk and Levene tests respectively. Seed survival curves were fitted using the *drc* R package for dose–response curves fitting that include different adjustments, including Weibull models that can contemplate asymmetrical losses' responses [62]. The best model was selected using the AIC criteria. Longevity parameters (L_5, L_{50} and L_{95}) were estimated using the ED function from the *drc* R package. SigmaPlot 10.0 (Systat, USA) was used to create the presented plots.

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Authors' contributions

EF and SMB conceived the idea and EF, LJ and SMB designed the experimental design. EF and LJ performed the experiments and analyzed the obtained data. EF prepared the manuscript. LJ and SMB revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Bekker RM, Bakker JP, Ozinga WA, Thompson K. Seed traits: essential for understanding seed longevity. Ann Appl Biol. 2003;69(1):1–9.
2. Dowsett C, James T, Trivedi P. Adaption of a technique for the accelerated aging of weed seeds to evaluate their longevity. N Z Plant Protect-SE. 2012;65:69–73.
3. Finch-Savage WE, Bassel GW. Seed vigour and crop establishment: extending performance beyond adaptation. J Exp Bot. 2016;67(3):567–91.
4. Company T, Soriano P, Estrelles E, Mayoral O. Seed bank longevity and germination ecology of invasive and native grass species from Mediterranean wetlands. Folia Geobot. 2019;54(1):151–61.
5. Pereira Lima JJ, Buitink J, Lalanne D, Rossi RF, Silva EAA, et al. Molecular characterization of the acquisition of longevity during seed maturation in soybean. PLoS ONE. 2017;12(7):e0180282.
6. Sallon S, Solowey E, Cohen Y, Korchinsky R, Egli M, Woodhatch I, Simchoni O, Kislev M. Germination, genetics, and growth of an ancient date seed. Science. 2008;320(5882):1464.
7. Bewley JD, Bradford K, Hilhorst H, Nonogaki H. Seeds. Physiology of development, germination and dormancy. 3rd ed. Berlin: Springer; 2013.
8. Hay FR, Valdez R, Lee JS, Sta Cruz PC. Seed longevity phenotyping: recommendations on research methodology. J Exp Bot. 2019;70(2):425–34.
9. Rajjou L, Debeaujon I. Seed longevity: survival and maintenance of high germination ability of dry seeds. C R Biol. 2008;331(10):796–805.
10. Long RL, Panetta FD, Steadman KJ, Probert RJ, Bekker RM, Brooks S, Adkins SW. Seed persistence in the field may be predicted by laboratory-controlled aging. Weed Sci. 2008;56(4):523–8.
11. McDonald MB. Seed deterioration: physiology, repair and assessment. Seed Sci Technol. 1999;27(1):177–237.
12. Powell AA, Don R, Haigh P, Phillips G, Tonkin JHB, Wheaton OE. Assessment of the repeatability of the controlled deterioration test both within and between laboratories. Seed Sci Technol. 1984;12:421–7.
13. Powell AA, Matthews S. Towards the validation of the controlled deterioration vigour test for small-seeded vegetables. Seed Test Int. 2005;129:21–4.
14. Delouche JC, Baskin CC. Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci Technol. 1973;1:427–52.
15. Hampton JG, Tekrony DM. Handbook of vigour test methods. Zurich: The International Seed Testing Association; 1995.
16. Walters C, Hill LM, Wheeler LJ. Dying while dry: kinetics and mechanisms of deterioration in desiccated organisms. Integr Comp Biol. 2005;45(5):751–8.
17. Marcos-Filho J. Seed physiology of cultivated plants. 2nd ed. Londrina: ABRATES; 2015.
18. Hay FR, Whitehouse KJ. Rethinking the approach to viability monitoring in seed genebanks. Conserv Physiol. 2017;5(1):9.
19. Newton R, Hay F, Probert R. Protocol for comparative seed longevity testing. In: Technical information Sheet_01. Millennium Seed Bank Partnership, Royal Botanic Gardens, Kew. 2014.
20. Maier DS. Should biodiversity and nature have to earn their keep? What it really means to bring environmental goods into the marketplace. Ambio. 2018;47(4):477–92.
21. Faith D. Biodiversity's option value: a comment on Maier. Ambio. 2018;47(6):735–6.

22. Maier DS. Response to "Biodiversity's option value: a comment on Maier (2018)" by Faith. Ambio. 2018;47(6):737–8.
23. Gioria M, Pyšek P. The legacy of plant invasions: changes in the soil seed bank of invaded plant communities. BioSci. 2016;66(1):40–53.
24. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Global assessment report on biodiversity and ecosystem services, IPBES. 2019.
25. Vilà M, Valladares F, Traveset A, Santamaría L, Castro P, editors. Invasiones Biológicas. Madrid: CSIC y Catarata; 2008.
26. Mudimba TN, Nguta JM. Traditional uses, phytochemistry and pharmacological activity of *Carpobrotus edulis*: a global perspective. J Phytopharmacol. 2019;8(3):111–6.
27. Vilà M, Tessier M, Suehs CM, Brundu G, Carta L, Galanidis A, et al. Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. J Biogeogr. 2006;33(5):853–61.
28. Vieites-Blanco C, González-Prieto SJ. Effects of *Carpobrotus edulis* invasion on soil gross N fluxes in rocky coastal habitats. Sci Total Environ. 2018;619–620:966–76.
29. Novoa A, González L. Impacts of *Carpobrotus edulis* (L.) NE Br. on the germination, establishment and survival of native plants: a clue for assessing its competitive strength. PLoS ONE. 2013;9(9):e107557.
30. Chenot J, Affre L, Passeti A, Buisson E. Consequences of iceplant (Carpobrotus) invasions on the vegetation and seed bank structure on a Mediterranean island: response elements for their local eradication. Acta Bot Gallica. 2014;161(3):301–8.
31. Cacho OJ, Spring D, Pheloung P, Hester S. Evaluating the feasibility of eradicating an invasion. Biol Invasions. 2006;8(4):903–17.
32. Panetta FD, Cacho O, Hester S, Sims-Chilton N, Brooks S. Estimating and influencing the duration of weed eradication programmes. J Appl Ecol. 2011;48(4):980–8.
33. Bailly C. Active oxygen species and antioxidants in seed biology. Seed Sci Res. 2004;14(2):93–107.
34. Kibinza S, Vinel D, Côme D, Bailly C, Corbineau F. Sunflower seed deterioration as related to moisture content during aging, energy metabolism and active oxygen species scavenging. Physiol Plantarum. 2006;128(3):496–506.
35. Lehner A, Mamadou N, Poels P, Côme D, Bailly C, Corbineau F. Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during aging in wheat grains. J Cereal Sci. 2008;47(3):555–65.
36. Yao Z, Liu L, Gao F, Rampitsch C, Reinecke DM, Ozga JA, et al. Developmental and seed aging mediated regulation of antioxidative genes and differential expression of proteins during pre- and postgerminative phases in pea. J Plant Physiol. 2012;169(15):1477–88.
37. Morscher F, Kranner I, Arc E, Bailly C, Roach T. Glutathione redox state, tocochromanols, fatty acids, antioxidant enzymes and protein carbonylation in sunflower seed embryos associated with after-ripening and aging. Ann Bot. 2015;116(4):669–78.
38. Zhou W, Chen F, Luo X, Dai Y, Yang Y, Zheng C, et al. A matter of life and death: molecular, physiological, and environmental regulation of seed longevity. Plant, Cell Environ. 2020;43(2):293–302.
39. Luna B, Moreno JM, Cruz A, Fernández-González F. Heat-shock and seed germination of a group of Mediterranean plant species growing in a burned area: an approach based on plant functional types. Environ Exp Bot. 2007;60(3):324–33.
40. Jaureguiberry P, Díaz S. Post-burning regeneration of the Chaco seasonally dry forest: germination response of dominant species to experimental heat shock. Oecologia. 2015;177(3):689–99.
41. Fichino BS, Dombroski JRG, Pivello VR, Fidelis A. Does fire trigger seed germination in the neotropical savannas? experimental tests with six Cerrado species. Biotropica. 2016;48(2):181–7.
42. Daws MI, Kabadajic A, Manger K, Kranner I. Extreme thermo-tolerance in seeds of desert succulents is related to maximum annual temperature. S Afr J Bot. 2007;73(2):262–5.
43. Whitaker C, Berjak P, Kolberg H, Pammenter N. Responses to various manipulations, and storage potential, of seeds of the unique desert gymnosperm, *Welwitschia mirabilis* Hook. fil. S Afr J Bot. 2004;70(4):622–30.
44. Suresh A, Shah N, Kotecha M, Robin P. Effect of natural, accelerated and saturated salt accelerated aging on the *Jatropha curcas* L. seeds in optimizing the yield of seed oil as feedstock for biodiesel. Energ Source Part A. 2019;41(8):990–1004.
45. Mira S, Veiga-Barbosa L, Pérez-García F. Seed dormancy and longevity variability of *Hirschfeldia incana* L. during storage. Seed Sci Res. 2019;29(2):97–103.
46. Wettlaufer SH, Leopold AC. Relevance of Amadori and Maillard products to seed deterioration. Plant Physiol. 1991;97(1):165–9.
47. Fu YB, Ahmed Z, Diederichsen A. Towards a better monitoring of seed aging under ex situ seed conservation. Conserv Physiol. 2015;3(1):cov026.
48. Yuan X, Wen B. Seed germination response to high temperature and water stress in three invasive Asteraceae weeds from Xishuangbanna, SW China. PLoS ONE. 2018;13(1):e0191710.
49. Zani D, Müller JV. Climatic control of seed longevity of Silene during the post-zygotic phase: do seeds from warm, dry climates possess higher maturity and desiccation tolerance than seeds from cold, wet climates? Ecol Res. 2017;32(6):983–94.
50. Fenollosa E, Munné-Bosch S, Pintó-Marijuan M. Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*. Physiol Plantarum. 2016;160(2):185–200.
51. Xu L, Lin Y, Wang L, Zhou YC. Dehiscence method: a seed-saving, quick and simple viability assessment in rice. Plant Methods. 2018;14:68.
52. Elias SG, Copeland LO, McDonald MB, Baalbaki RZ. Seed testing—principles and practices. East Lansing: Michigan State University Press; 2012.
53. Kranner I, Minibayeva FV, Beckett RP, Seal CE. What is stress? Concepts, definitions and applications in seed science. New Phytol. 2010;188(3):655–73.
54. Cohen O, Riov J, Katan J, Gamliel A, Bar P. Reducing persistent seed banks of invasive plants by soil solarization—The case of *Acacia saligna*. Weed Sci. 2008;56(6):860–5.
55. Cohen O, Gamliel A, Katan J, Kurzbaum E, Riov J, Bar P. Controlling the seed bank of the invasive plant *Acacia saligna*: comparison of the efficacy of prescribed burning, soil solarization, and their combination. Biol Invasions. 2018;20(10):2875–87.
56. Cohen O, Bar P, Gamliel A, Katan J, Kurzbaum E, Weber G, Schubert I, Riov J. Rain-based soil solarization for reducing the persistent seed banks of invasive plants in natural ecosystems—*Acacia saligna* as a model. Pest Manag Sci. 2019;75(7):1933–41.
57. Daws MI, Burslem DFRP, Crabtree LM, Kirkman P, Mullins CE, Dalling JW. Differences in seed germination responses may promote coexistence of four sympatric *Piper* species. Funct Ecol. 2002;16(2):258–67.
58. Hadley N. Micrometeorology and energy exchange in two desert arthropods. Ecology. 1970;51(3):434–44.
59. Patil VN, Dadlani M. Tetrazolium test for seed viability and vigour. In: Handbook of seed testing, 2009. pp. 209–41.
60. Novoa A, González L, Moravcová L, Pyšek P. Constraints to native plant species establishment in coastal dune communities invaded by *Carpobrotus edulis*: implications for restoration. Biol Conserv. 2013;164:1–9.
61. Podda L, Santo A, Mattana E, Mayoral O, Bacchetta G. Inter-and intra-variability of seed germination traits of *Carpobrotus edulis* NE Br. and its hybrid *C. affine acinaciformis*. Plant Biol. 2018;20(6):1059–67.
62. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-response analysis using R. PLoS ONE. 2015;10(12):e0146021.

Publisher's Note

Annex 4. Plasticitat fisiològica de les plantes per fer front al canvi climàtic

Annex 4. Physiological plasticity of plants facing climate change.

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PHYSIOLOGICAL PLASTICITY OF PLANTS FACING CLIMATE CHANGE

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Abstract: Plant plasticity is essential to provide plants with the most adequate physiological mechanisms to face climate stochasticity. Both biodiversity conservation and food security lie on our current understanding of physiological plasticity to project the impacts of climate change and establish mitigation strategies. In this article, we focus on the evolutionary role of plasticity, its costs and limits, molecular basis and the physiological mechanisms allowing the expression of the most adequate plant phenotype in response to the major current climate change drivers. Furthermore, we provide novel insights into the most common approaches and methods used to study and evaluate plasticity, using plant photoprotection as an example. It is concluded that much progress has been made in the role of plasticity in plant responses to climate change, although further insights into stress memory, sensory mechanisms, and the plant responses to multiple environmental stresses is still required to better understand plant responses to climate change.

Keywords: adaptation, climate change, fitness, memory, phenotypic plasticity, stress

1 What Is Physiological Plasticity and Why It Matters?

Life started in the Earth between 3900 and 3500 million years ago (Dodd et al., 2017). It has been a long time suffering vast environmental heterogeneities, from changes in the atmosphere composition, to changes in radiation, temperature and water availability regimes (Nakashima et al., 2018). Life has

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been forced to change together with the environmental changes under the dichotomy of adapting or going extinct (Rubio de Casas et al., 2009). Indeed, adaptation to environmental changes have been brought by different processes operating at different scales that interact with each other: natural selection and plasticity. Despite both having received considerable attention by researchers working in different areas, the role of plasticity is still an open debate issue, mainly due to the difficulties of performing empirical studies that help us better understand its nature and biological significance. *Phenotypic plasticity* is defined as the capacity of a genotype to produce different phenotypes (observable characteristics including morphology, growth and development, biochemical attributes, and/or physiological properties), maximising or not the species capacity to perform (grow and reproduce successfully) in a determined habitat. This concept is indeed related to the capacity of the species to grow and reproduce successfully, what we define as '*species fitness*'.

The current interest in plasticity results from an urgency to predict species responses to global change (Valladares et al., 2006). For a long time, plasticity was considered a nuisance in evolutionary studies. Decades of studies have revealed that plasticity is a property of the genotype, a trait itself, playing a crucial role in species adaptation to environmental conditions, providing a buffer against rapid climate changes and to assist rapid adaptation (Lande, 2009; Chevin et al., 2010). Therefore, plasticity may allow survival of populations that as the climate changes may otherwise no longer be optimally adapted to new conditions and they will be forced to migrate or becoming extinct. The importance of phenotypic plasticity in plants relies on the fact that as sessile organisms, during their post-embryonic stage, they face variability in environmental conditions once they become established after germination. Phenotypic plasticity is also determinant during the colonisation of a new habitat or when the global environmental framework shifts (climate change). Species distribution may in part be determined by their phenotypic plasticity, as it has been shown in different species of *Cistus*, where phenotypic plasticity correlates positively with a wider ecological distribution (Catoni et al., 2012). Moreover, plasticity provides a buffer period during which niche evolution (i.e. adaptive niche expansion or shift) might occur (Chevin et al., 2010). For example, the survival of species such as *Quercus coccifera* during glaciations on the quaternary has been attributed to a high phenotypic plasticity under suboptimal conditions (Rubio de Casas et al., 2009). Evolution of plasticity is indeed possible thanks to the occurrence of variability due to both genetic and environmental variation, and in particular, the interaction between them (the so-called genotype x environment, or GxE interaction). An individual or a population is plastic when GxE interaction is consistently expressed. In this article, we review the state of the art of phenotypic plasticity with a particular focus on the physiological mechanisms underlying it. Furthermore, we focus on the

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plasticity of physiological responses to climate change drivers, collecting empirical evidence of the plastic changes and plastic mechanisms in plants facing global change. The understanding of the mechanistic basis of plant plastic responses to climate change drivers is essential to predict adaptation of the different species to a changing climate.

2 Towards the Adaptive Role of Phenotypic Plasticity

Plasticity can be both adaptive and nonadaptive (Ghalambor et al., 2007). Plasticity is adaptive if the phenotype produced in response to a change in the environment results in an improved fitness (Van Kleunen and Fischer, 2004). The resulting reaction norm is in the same direction as the optimal value favoured by selection in the new environment (Ghalambor et al., 2007). Adaptive plasticity enhances the probability of persistence in a new environment and can facilitate adaptive genetic differentiation when directional selection acts on extreme phenotypes (Price et al., 2003). It confers greater tolerance to changing conditions (Ghalambor et al., 2007), either by enabling species to tolerate new environments and persist long enough to adapt or by directly facilitating evolution through genetic assimilation (West-Eberhard, 2005). Adaptive plasticity may be advantageous when it allows a genotype to have a broader tolerance to environmental conditions and hence higher fitness across multiple environments. Adaptive plasticity can be classified as perfect or incomplete (Ghalambor et al., 2007). A perfect adaptive plastic response occurs when the mean phenotype is a close match to what is favoured by selection in the new environment, whereas an incomplete response occurs when adaptive plasticity produces a mean phenotype that is in the direction of the optimum favoured by selection, but the match is incomplete. An example of adaptive plastic response under climate change is the CO_2 fertilisation effect (CFE), an increased plant metabolic performance due to increased photosynthesis substrate in the atmosphere (Long et al., 2004).

The definition of adaptive plastic responses depends clearly on the definition of fitness, leading to some contradictory conclusions when considering first sight nonadaptive responses. Plastic responses that do not maximise the capacity of a species to survive, reproduce and propagate in a given environment are theoretically defined as nonadaptive plastic responses. However, are we confident that nonadaptive plasticity is useless for species survival? Despite some plastic responses not maximising the species fitness in some environments, they may do in others. Nonadaptive plasticity may not be an undesirable result for a species in a certain environmental framework. Nonadaptive plastic responses that promote stress tolerance may permit plant persistence under suboptimal conditions and may be

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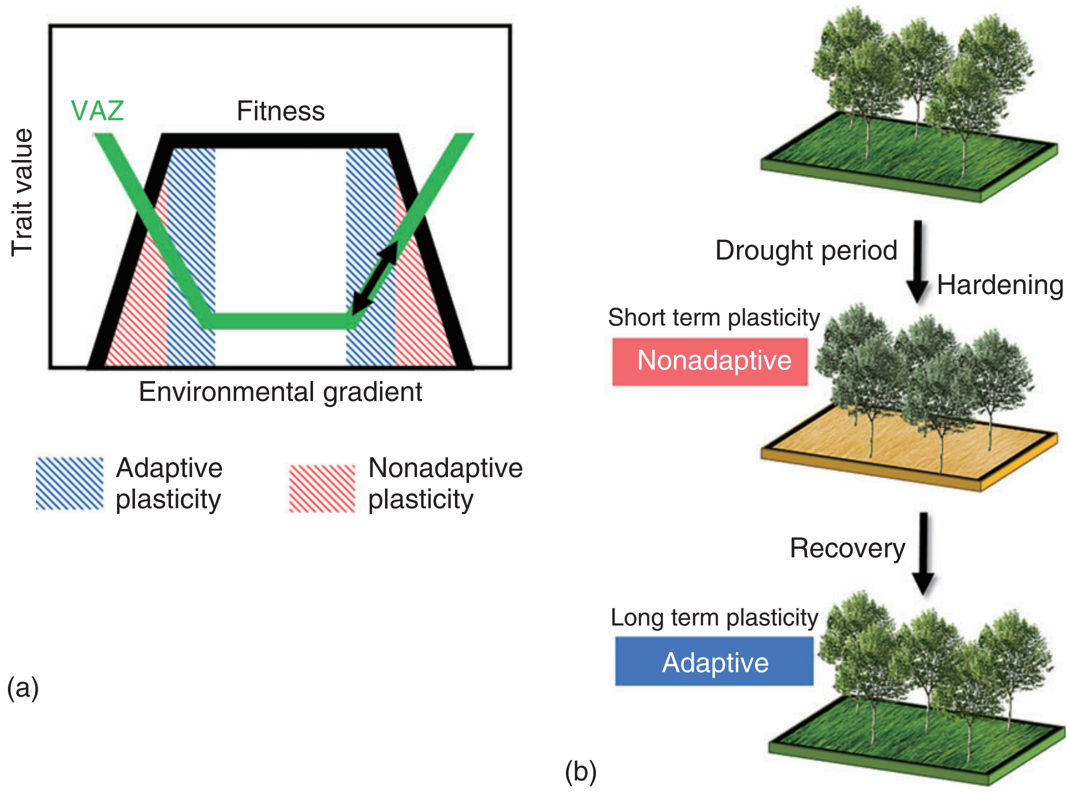

Figure 1 Towards the adaptive meaning of phenotypic plasticity: (a) The role of stress intensity and (b) the role of the evaluation time. VAZ: total xanthophyll pool, as an example of a stress-responsive plastic trait that may be adaptive on a certain range along environmental axis.

more adaptive than nontolerant or nonreversible responses. Therefore, the extent of the considered benefits may be crucial to determine the adaptive role of plastic changes (Figure 1). Whether plasticity is adaptive or not is dependent upon the environment in which it is expressed but also on the future environmental regime (Mikolajewski et al., 2006). Despite certain plasticity being defined as nonadaptive at a given environment for a given genotype in a given developmental stage, it may be adaptive when any of the cited elements defining the framework changes. Nonadaptive plastic changes can potentially increase future fitness, depending mainly on the future environmental conditions. The attribution to nonadaptive or adaptive to a plastic response has become a difficult aspect to evaluate because instantaneous fitness may not reflect the adaptive role of the plastic response. Furthermore, the categorisation of adaptive and nonadaptive plant responses may not be universal. Despite some authors pointing out that nonadaptive plasticity may not occur as it may not be naturally selected, not all the processes driving evolution require natural selection. Maladaptive or nonadaptive genetic changes can occur during genetic drift or gene flow, rather than natural selection (Merilä and Hendry, 2013).

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2.1 Stress Resistance and Reversible Plasticity

Plants have different strategies to adapt to the prevailing environmental conditions out of their optimal range. Avoidance includes strategies that prevent the external stress factor from triggering responses that modify plant functioning. Annual plants are experts on stress avoidance, as their cycle is confined to the optimal conditions period, escaping the harsh season. In contrast, tolerance mechanisms are those that permit the plant to withstand stress, so that the plant physiology is altered to face and adequately respond to stress. Plasticity appears in each of the different mechanisms. But are all stress responses adaptive or are they just an unavoidable consequence of the impairment of some vital plant functions? Regarding this consideration, plasticity can be divided into *active* and *passive plastic responses*, where active responses are those that are used predominantly as anticipatory phenotypic changes and passive responses represent the direct influences of the environment (Forsman, 2015). Observed response may be a summary of both passive and active plasticity. The empirical distinction of passive and active plasticity is however almost impossible. Passive plasticity can be associated with sensitivity to certain environmental conditions. Under stress conditions, a plastic genotype can build a different phenotype but may not increase the final fitness. Finally, we can distinguish between *sensitive* and *tolerant genotypes* (Figure 2), or sensitive and tolerant traits. In some cases, under stressful conditions, canalisation (i.e. the lack of plasticity) is the best hope for increasing the likelihood of persistence in the new environment. However, canalisation

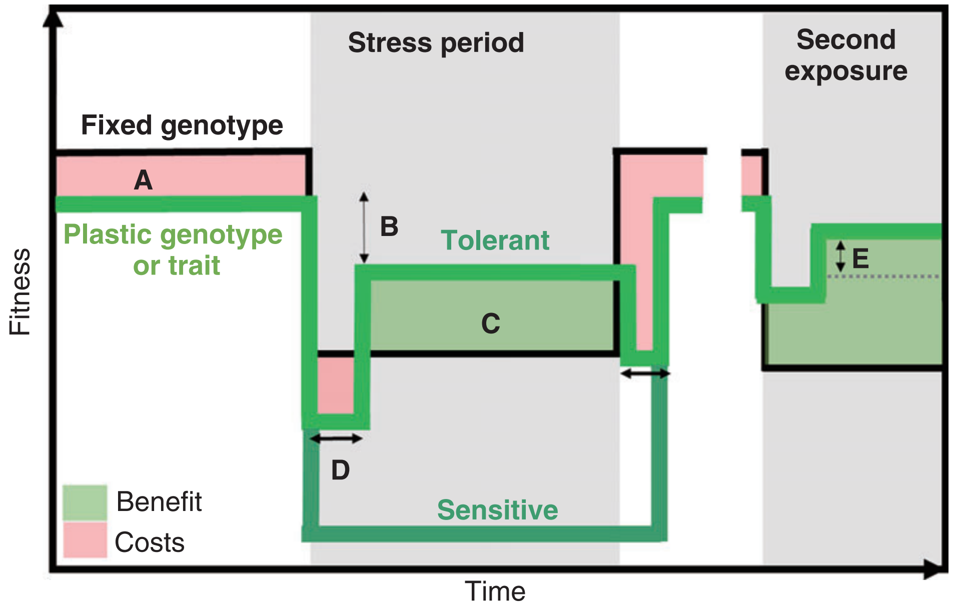

Figure 2 Limits, costs, and benefits of plasticity under stress conditions. A, plasticity costs; B, plasticity limits; C, plasticity benefits; D, lag-time; E, memory. Source: Modified from Gabriel (2005).

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may be possible due to an extreme plasticity in some other traits, allowing the plant to have fitness homeostasis. Canalisation has occurred due to breeding in many crop species, especially in yield-related traits (Dalal et al., 2017).

Some stress responses have been described as *reversible plasticity*. Plasticity occurring reversibly through life is *acclimation*. If acclimation increases the fitness under a second exposure (regarding individuals experiencing a first stress exposure) of the stressful environmental conditions, we talk about *memory* (Figure 2E). Another important term regarding memory events is *hardening or priming*, which is the process of endurance with a soft or first stress that increases the ability to respond later to those stressful conditions. The plastic response may not occur immediately after exposure to environmental cues, as well as its potentially adaptive benefits, due to time-lag responses and costs (Figure 2). Stress and recovery have costs and limits, which are assumed to be symmetric, as well as exhibiting time lags (Gabriel, 2005). Despite plasticity under stressful conditions correlating with plasticity in *Cistus* recovery (Puglielli et al., 2017), some traits may have asymmetric or hysteretic responses. During the last decade, more emphasis has been put on understanding plant memory mechanisms which has even led to the new proposed field of Plant Gnosophysiology, as suggested by Michmizos and Hilioti (2019). Different mechanisms may explain memory in plants, such as sustained levels of key signalling metabolites or transcription factors or alterations in chromatin states (histone tail modifications, DNA methylation or paused RNA polymerase II) (Crisp et al., 2016). Nonadaptive plastic responses can be suppressed by a memory and learning process. *Mimosa pudica* has a leaf folding defensive strategy against predation, but this mechanism is also activated by other mechanical disturbances. *Mimosa* learns to suppress this response when it is nonadaptive, as in the case of a series of drops that are not associated with predation (Gagliano et al., 2014). Memory in the sensory plant mechanisms can allow a learning process to appropriately adjust the phenotype to the environment, reducing possible mismatches (Bigot et al., 2018). Hardening or priming that persist between generations is called *adaptive transgenerational plasticity* (Herman and Sultan, 2011). Under a drought priming treatment, *Polygonum persicaria* progeny presents increased root growth and biomass as well as improved drought tolerance (Sultan et al., 2009). Not only memory to different environmental conditions but also memory at the ecosystem level may modify the impact of climate change (Hughes et al., 2019).

3 Costs and Limits of Physiological Plasticity

Because no organism is infinitely or ideally plastic, theory suggests that there must be some restrictions to phenotypic plasticity. The existence of reversible

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plasticity also evidences that at least a maintenance cost may be associated. Two types of restrictions may exist: costs and limits.

3.1 Costs

Costs of phenotypic plasticity are an important restriction determining the evolutionary role of plasticity (Figure 2). Costs constitute a decrease in fitness even when an optimal phenotype is expressed. Costs appear when we compare a plastic with a fixed genotype in a given environment and compare fitness over the same trait value (Van Tienderen, 1991). The costs of plasticity can be an aggregation of different elements. For instance, the costs of a phenotype are different from the costs of plasticity (Callahan et al., 2008). A cost of plasticity refers to the fitness decrement of a highly plastic genotype relative to a less plastic genotype due to the costs associated with carrying around additional genetic machinery (Knight et al., 2005). A cost of phenotype refers to the fitness trade-offs inherent in allocating resources to one trait vs. another, as well as the costs of obtaining information on the environment. Unfortunately, costs of phenotype and costs of some complex characters may be difficult to differentiate (Murren et al., 2015). In relation to this separation, some authors (Ernande and Dieckmann, 2004; Van Kleunen and Fischer, 2004) classify the costs in:

- *Maintenance costs*: The costs of acquiring and maintaining the sensory systems and the regulatory machinery required to collect information for plastic development. They occur even if a plastic phenotype is not expressed.
- *Production costs:* The costs of producing the phenotype. They only occur when a plastic phenotype is expressed. It also includes the costs of development instability.
- *Genetic costs:* The costs caused by negative genetic correlations as a consequence of pleiotropy, or of genetic linkage with deleterious alleles at other loci, or epistasis involving genes relevant for variation in fitness and plasticity.

Costs may do not always have the same importance. Net costs depend on: (i) the fitness benefit of plasticity, (ii) the frequency at which different types of environment occur, (iii) the quality and abundance of resources invested, and (iv) the sensitivity to phenotypic maladaptation in each type of environment (Ernande and Dieckmann, 2004). Costs of plasticity in response to plant density, light intensity, and light spectral composition have been observed for morphological and phenological traits of *Arabidopsis thaliana* (Dorn et al., 2000) and are more likely to occur under resource limitation. Moreover, costs may depend on the development stage and ontogeny. Early costs during development may determine the genotype success. Furthermore, some authors have quantified costs and pointed out that the costs of carrying unexpressed genes or additional regulatory elements is likely to

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be negligible in most cases (Latta et al., 2012). But the role of phenotypic plasticity costs in adaptation is still an open debate, mainly due to the lack of empirical evidence with various species.

3.2 Limits

Limits to plasticity can cause plants to fail in producing the optimal phenotype for a given environment (Pigliucci, 2005). From a general point of view, limits to plasticity come from (i) the capacity for immediate change, and (ii) the production of the optimal phenotype (Van Kleunen and Fischer, 2004). Valladares et al. (2007) classified the limits into internal (genetic costs, allocation, ontogeny, plasticity history, development constraints, phenotypic integration and lag-time of the response) and ecological limits (reliability of environmental cues, abiotic stresses, herbivory, competition, multispecies interactions, and multiple simultaneous stresses). Genetic costs, for example, limit phenotypic plasticity due to genetic correlations between any two phenotypic traits. Genetic costs can be estimated with the proportion of phenotypic variance statistically associated with genetic differences among individuals. In relation to genetic costs, genetic architecture can constrain their plastic, functional responses to the environment. In that way, the number of significant correlations between traits (phenotypic integration) decreases with phenotypic plasticity (Gianoli and Palacio-López, 2009). It has been demonstrated that plant history and developmental stage limits plant future plastic responses. Phenotypic plasticity may not be constant during plant development (Murren et al., 2015), and development itself can be viewed as a continuous reaction norm of the entire genotype. The plant development process may involve the sampling of a range of phenotypes through environmental feedback reinforcing high-performance phenotypes. This trial-and-error learning increases the probability that an individual will develop a phenotype best matched to the local environment (Snell-rood and Carolina, 2012). Development modifiers (regulatory genes or proteins that affect the contribution of other genes to the phenotype) reduce costs of acclimation (Beaman et al., 2016). However, on the other side of the coin, environmental feedback can be costly, increasing production costs. It is crucial to integrate development into models of species' responses to changing environments. In that way, the sensory mechanisms and the reliability of environmental cues (the likelihood of environmental change) may determine the plastic change. Abiotic and biotic stresses can also limit plasticity either because the potential plastic response in a given trait cannot be fully achieved due to resource limitation, or because expressing plasticity would make organisms more vulnerable to stress (Valladares et al., 2007).

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4 Molecular Basis of Physiological Plasticity

Research in phenotypic plasticity has sometimes progressed ignoring the details of the genes underlying a reaction norm (Callahan et al., 2008). However, understanding the plant response under changing environments requires a better comprehension of the machinery underlying plasticity: structural and regulatory genes, sensory proteins, and biochemical pathways. Heritable and nonheritable environmentally induced variation has been described in explanations of phenotypic plasticity. Heritable phenotypic variation does not need to be based on DNA sequence polymorphism (Richards, 2006; Bossdorf et al., 2008). Heritability of plasticity is also attributed to overdominance/homozygosity, pleiotropy, and epistasis (Scheiner, 1993). Considering this process, two main models have been proposed to describe genetic mechanisms underlying plastic responses: allelic sensitivity and epistasis. The first model, often cited as allelic sensitivity or pleiotropic model, postulates that phenotypic plasticity is a function of differential expression of the same genes under a different environment (Avolio et al., 2018). The second model, gene regulation or epistasis model, establishes that phenotypic plasticity originates from the interaction between genes that determine the magnitude of responses to environmental effects and genes that determine the average expression of the character (Scheiner, 1993). Recently, Van Heerwaarden and Sgrò (2017) postulated that models that ignore epistasis may be insufficient to understand and predict evolutionary responses to climate change. On the other hand, some important traits such as flowering time and seed germination are regulated pleiotropically in *A. thaliana* (Auge et al., 2018).

Besides the exposed genetic mechanisms for explaining plasticity, increasing evidence of transgenerational epigenetics and their role on individual plasticity has been experimentally gathered during the last decade (Crisp et al., 2016). Epigenetic processes include DNA methylation, histone modification, chromatin remodelling, and transposable element activation. Although epigenetic modifications can be stochastic, there is evidence that they can be induced in response to stress and environmental changes both in controlled and in natural conditions (Balao, 2018). After 12 years of partial rain exclusion, *Quercus ilex* trees differentiated epigenetically but not genetically (Rico et al., 2014). Moreover, it has been shown that water deficiency induces pericentromeric DNA hypermethylation enhancing root growth in *A. thaliana* (Virdi et al., 2015).

The process by which nonheritable environmentally induced variation leads to adaptive heritable variation is often referred to as the Baldwin effect or more commonly as *genetic assimilation* (Ghalambor et al., 2007). Genetic assimilation is the environmentally induced canalisation or loss of plasticity of some traits. West-Eberhard (2005) advocates for a less restrictive term: genetic accommodation, which does not necessarily imply a loss in plasticity.

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However, a recent modelling of the genetics of plasticity (Scheiner et al., 2017) found that genetic assimilation or accommodation is not likely to occur because the replacement of plasticity by fixed genetic effects takes much longer than the environment is likely to remain stable.

One approach to understand the role of genetics in determining individual plasticity is to study the genetic architecture, the number and type of loci that determine heritable variation in trait phenotype, distinguishing between plastic and nonplastic loci (whose expression is not affected by the environment) (Scheiner and Holt, 2012). Genetic architecture, the genes that underlie the plastic responses, their number, chromosomal locations, and genetic interactions as well as environmental impact, affect the evolutionary outcome (Wang et al., 2012). The study of the genetic architecture together with the development of new molecular techniques such as linkage disequilibrium mapping, high-throughput sequencing, microarrays, proteomic analysis, genome-wide association studies (GWAS), candidate gene approaches, and allele-environment correlations are allowing researchers to probe deeply into the understanding of the molecular basis of physiological plasticity.

5 Environmental Sensing and Signalling by Plants

Environmental cues are biotic and abiotic factors that induce phenotypic plasticity, including environmental, chemical, or hormonal cues (Turcotte and Levine, 2016). The expression of a phenotype is driven by different mechanisms that operate at different hierarchical levels. Before a phenotype changes, the environmental cue is perceived by the plant, transduced and signals transmitted. The resilience or the hypersensitivity of the sensing and signalling mechanisms is a determinant in the expression of an adaptive phenotype avoiding maladaptive responses to face climate change. Sensing and signalling mechanisms for phenotypic plasticity have been generally considered a black box, but during the last decade, research has led to the possibility of integrating these mechanisms into the study of species response to climate (Bigot et al., 2018). Some of the most important plant sensors, internal messengers, and signal transduction pathways are summarised here.

One of the recent important discoveries on the sensing mechanisms in plants is the double role of *phytochrome B* (phyB), which has been recently presented as an essential thermal sensor aside from its well-known role in light quality and intensity perception (Jung et al., 2016; Legris et al., 2016). During the day, the inactive Pr form of phyB is converted by red light into active Pfr form. This conversion is reversed by far-red light but also by high temperatures by a process called dark or thermal reversion. The balance between Pr and Pfr pools act as an internal clock. Pfr binds to

PIF (phytochrome-interacting factor) transcription factors and regulate the transcription of target genes. In *A. thaliana*, PIF4 is regulated by phyB thought sequestration and degradation, and it is essential for temperature-dependent responses (Koini et al., 2009). PhyB and PIF4 are involved in primary cell wall plasticity, cell elongation, leaf cell differentiation into stomata, cell division, hypocotyl elongation, stomatal index regulation, and flowering time (Bigot et al., 2018).

Besides phyB, *reactive oxygen species* (*ROS*) including singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$) constitute a major hub in the response to different abiotic stresses and have been proposed to play a major signalling mechanism triggering plant stress responses (Apel and Hirt, 2004). Importantly, ROS generation in cellular compartments such as the mitochondria or chloroplasts results in changes of the nuclear transcriptome, a process known as retrograde signalling (Rentel et al., 2004).

Also, the amount of intracellular Ca^{+2} is involved in a systemic plant response through the recently described ‘calcium waves’, which may run long distances within an individual (Choi et al., 2014). Intracellular calcium increases in response to H_2O_2 and lead to changes in cell turgor *via* the activation of selective ion channels. The production of H_2O_2 from NADPH is catalysed by NADPH oxidase which require Ca^{+2}, suggesting a possible feedback mechanism between calcium signalling and ROS production. In this way, the *gca2 A. thaliana* mutant shows impaired ROS activation of guard cell Ca^{+2} permeable channels and it is insensitive to high CO_2 (Pei et al., 2000). Moreover, Ca^{+2} triggers the activation of heat shock transcription factors (Saidi et al., 2010). Under stress conditions, the generated ROS triggers an ‘oxidative burst’ leading to a systemic response that has been described under mechanical damage, low temperatures, light stress and salinity (Miller et al., 2009). The cellular redox state has also been proposed to act as a signalling sensor mechanism for several stresses (Munné-Bosch et al., 2013). Some oxidised products are known to be responsible for some plant stress responses. For instance, β-cyclocitral (an oxidised product of β-carotene) acts as an alarm signal inducing retrograde signalling from chloroplasts to the nucleus (Ramel et al., 2012).

Electrical signals at the plasma membrane level have also been proposed as key players in long-distance signalling, especially in response to wounding and cold, but more research is required to understand the mechanisms of the transmission of action potentials in plants (Hedrich et al., 2016; Edelaar et al., 2017).

Phytohormones, including abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), auxins, cytokinins (CKs), gibberellins (GA), and ethylene (ET) also constitute key internal signals to develop the most appropriated responses under the prevailing environmental conditions. ABA, JA, SA, and ET play major roles in plant responses to biotic and abiotic stresses (Verma

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et al., 2016). GA, auxins, and CKs are involved in the regulation of plant development and therefore are key players in the determination of plant morphological plasticity. Leaf morphology, for instance, determines plant capacity to acquire light and regulate water loss. Heterophylly, or the ability to alter leaf form in response to environmental conditions, is an adaptive mechanism strongly regulated by a coordinated action between ABA, ET, GA, and auxins (Nakayama et al., 2017).

The possibility to develop accurate models to predict plant responses to climate change relies on our capacity to unravel the sensory machinery and signal transduction pathways. For instance, the model developed by Colautti and Barrett (2013) suggests that *Lythrum salicaria* expansion in North America is possible thanks to plasticity in flowering time. However, the assessment of which environmental cue a species may exhibit plasticity towards is challenging, and climate change predictions may differ strongly if *L. salicaria* is plastic for example in response to photoperiod or temperature. Complexity in understanding plant responses to environmental cues may also be due to redundancy in signalling machinery as in the case of stomatal regulation or auxin production and transport (Brumos et al., 2018). Moreover, little differences in exposure time, stress severity, or light intensity can lead

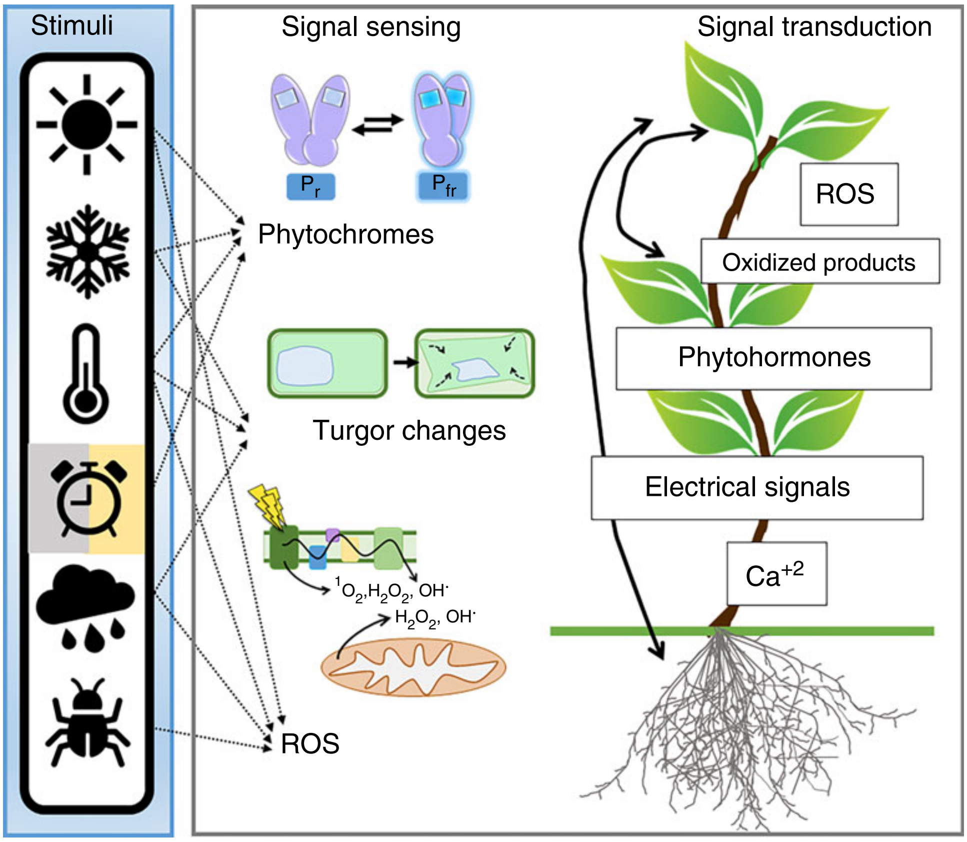

Figure 3 Some of the major stress sensing and signalling pathways accounting for plastic responses in plants.

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to different signalling pathways, as in the case of the hormonal response of *Carpobrotus edulis* to chilling (Fenollosa et al., 2018) or the *Saccharum* spp. morphological and physiological drought response (Marchiori et al., 2017). Furthermore, multiple environmental stressors occurring together determine ultimately the response of a plant to conditions of climate change, leading to complex and sometimes unknown signal interactions (Coté et al., 2016). Synergistic or conflicting sensing and signalling outcomes can arise in response to complex environmental cues, but not all environmental heterogeneity may be functionally relevant for the plant (Bigot et al., 2018). More research should be conducted to understand relevant and reliable cues for plants that lead to biologically significant plastic changes in response to climate change drivers (Figure 3).

6 Plant Plasticity and Climate Change

The last decades have revealed overwhelming evidence that the world's climate is changing. Warming of the climate system due to global human activities is unequivocal, and many of the changes that have been observed this century are unprecedented. The atmosphere and ocean have warmed, the amounts of snow and ice diminished, and sea level has risen, according to the fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC 2014). Despite the uncertainty associated with predicting the future, atmospheric CO_2 increase, temperature increase, and changes in the water distribution are the most certain and major effects that can potentially threaten plant productivity. An increase in extreme events is also predicted, including both heavy precipitation and high temperatures (Fischer et al., 2018). Not only the magnitude but the rate of changes is threatening all species. The unusual rate and extent of anthropogenic alterations of the environment may exceed the capacity of developmental, genetic, and demographic mechanisms that populations have evolved to deal with environmental change. Responses to climate change are likely to be highly dependent on the rate, scale, and predictability of environmental changes.

6.1 Responses to Plants to CO_2, Temperature, and Drought

Since the era of industrialisation, atmospheric CO_2 has been increasing from 280 ppm during the pre-industrial era to 390 ppm until 2013 (IPCC 2014). The last decades have brought new technologies to evaluate the effects of CO_2 enrichment under natural conditions such as open-top chambers (OTC) or free-air CO_2 enrichment (FACE) (Hendrey et al., 1999). In 156 experiments with five different methods to increase CO_2 (such as FACE, OTC, etc.),

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doubling CO$_2$ levels from 350 to 700 ppm increased yield approximately 31% (Amthor, 2001). This results from increased net photosynthesis and photorespiration inhibition and it is known as the 'CO$_2$ fertilisation effect' (Long et al., 2004). Despite this effect has been proven in several studies, elevated concentrations of CO$_2$ regulate in the short term the activity of different ion and organic solute channels altering its concentration in the guard cells, reducing stomatal aperture (Raschke et al., 2003). A longer-term effect of the continuing CO$_2$ rise is the downregulation of stomatal development in the leaf epidermis (Woodward, 2002). However, not all studies reach the same conclusions, and it is hypothesised that stomatal density in response to high CO$_2$ may depend on species, leaf age, and plant growth conditions (Woodward et al., 2002).

Moreover, increased CO$_2$ responses depend on the plant metabolism type. Differences between C$_3$ and C$_4$ plants have been reported in response to CO$_2$. These are due to the inefficiency of RuBisCO and its contribution to photorespiration in C$_3$ plants, and the consequential benefit of an increase in CO$_2$ concentration reducing photorespiration. In C$_4$ plants, the first enzyme that incorporates CO$_2$ into a 4-carbon intermediate (phosphoenolpyruvate carboxylase, PEP) has a high affinity for CO$_2$, and therefore increased CO$_2$ concentrations have little impact on photosynthetic rate in these species. After some years, we are now able to evaluate the effects of enhanced CO$_2$ concentration on plants over a longer term. For example, a 20-year field experiment revealed that, despite C$_3$ plants exhibiting higher biomass production under enhanced CO$_2$ during the first 12 years, as expected, no effect was observed for C$_4$ plants. However, during the subsequent eight years, this situation reversed with a marked biomass increase in C$_4$ plants under high CO$_2$ (Reich et al., 2018). Nievola et al. (2017) have attributed this reversed pattern to interannual climate variation and highlights the complexity of predicting short and long-term CO$_2$ responses.

Morphological plasticity or structural adjustments have been described in plants under high CO$_2$ concentrations. CFE is assumed for some species in response to high CO$_2$, but the consideration of carbon allocation to different plant organs is crucial to understand the adaptive or nonadaptive role of CFE (Sevanto and Dickman, 2015). For example, rice grown under 600 ppm of CO$_2$ showed thicker culm wall of basal internodes due to elevated starch deposition in the stem, reducing lodging susceptibility (Zhao et al., 2019). However, some authors have observed a down-regulation of photosynthesis in the long term as a consequence of insufficient plant sink capacity (Aljazairi and Arias, 2015). This CFE limitation may also be attributed to nutrient availability, as rising CO$_2$ alters plant C-N-P stoichiometry (Du et al., 2019).

A second major driver of plant adaptation to climate change is air *temperature*. Global temperature is predicted to increase from 1.5 to 2.5 °C by the end of the twenty-first century (IPCC 2014). Temperature is a major environmental constraint for species distribution as it limits plant growth

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(Mittler, 2006), and it has important consequences for crop production. Wheat yields are predicted to decrease by circa 6% for every 1 °C rise in global temperature (Asseng, 2015). Photosynthesis itself is the most temperature-sensitive metabolic pathway in plants both at low and high temperatures, due to the high sensitivity of the photosystem II (PSII) to temperature increases (Mathur and Jajoo, 2014). Cell membranes are highly affected by temperature, altering their fluidity and perturbing ion transport, eventually leading to cell rupture and death. Increased oxidative stress and ion leakage has been described in response to high temperatures, mainly due to membrane lipid peroxidation (Narayanan et al., 2016). Despite high temperatures having a constraining impact on plant growth, some species may benefit from this shift. Among 55 cacti species, it is anticipated that up to 75% would have exhibited a significant increase in seed germination under projected climate change temperatures (Seal et al., 2017). Thermotolerance acquisition occurs after a previous exposure to low or high temperatures that cause no fatal injuries. After this hardening or priming effect, plants can withstand higher or lower temperatures that were previously considered harmful (de Pinto et al., 2015). Leaf thermic balance is mainly driven by the equilibrium between irradiance and the latent heat of vaporisation of water when stomata are open. Under increased temperature, stomatal opening may reduce leaf temperature but may also lead to plant dehydration if water is scarce. Structural adaptations play an important role in the response to increased temperatures, and responses such as wilting, paraheliotropism, or reflective leaf surfaces are effective strategies to alter the leaf thermic balance by decreasing leaf irradiance or absorbed energy. A list of metabolic adjustments to acquire thermotolerance in different plants is provided by Nievola et al. (2017) and it includes alterations in cell membrane composition, increased gene expression of heat shock proteins (HSPs), as well as enhanced production of chaperones and antioxidant activity. At 35 °C, wheat showed an increase in the proportion of saturated fatty acids with a major shift in the lipid composition, which avoids the fluidisation of the membranes (Narayanan et al., 2016). HSPs are produced by the plant after abrupt temperature changes of 5 °C (or more) above its optimum growth temperature, acting as chaperones protecting other proteins. The accumulation of antioxidant molecules has also been described for several species in response to increased temperatures (Nievola et al., 2017).

Some reproductive processes and vital events are especially sensitive to heat. Menzel et al. (2006) revealed that 78% of all plant phenological records analysed for 21 European countries showed a significant advancement in the occurrence of phenological events for the period 1971–2000. Records showed that as much as 30% of flowering and fruiting times occurred earlier during this period, with an average advance of 2.5 days per decade in Europe. Recent studies analysing long-term (1952–2013) effects of temperature on phenology

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of different cultivars of winter wheat also revealed alterations in phenology, identifying differential responses for the different cultivars (Rezaei, 2018).

A third major driver of plants adaptation to climate change is *drought* or water regime alteration. It is still not clear the direction of changes regarding global precipitation under climate change conditions, but several modelling attempts have revealed increased precipitation variability for most areas, increasing the number of drought events (Sheffield et al., 2012). Increased temperatures can enhance plant evapotranspiration and soil water evaporation, exhausting soil reserves. Different species may exhibit differentiated strategies facing low water availability, categorised as desiccation postponement, desiccation tolerance or drought escape. Resurrection plants are one of the most interesting examples of desiccation tolerance. They are capable of surviving the loss of 80–95% of their cell water content and yet can resume normal metabolic function on rehydration (Tuba, 2008). This ability, reduced only to a few plant groups (Lüttge et al., 2011), is the maximum expression of a desiccation tolerance strategy. Morphological adaptations include narrow leaves, increased mesophyll compactness, the existence of water storage tissue and sclerenchyma, abundant palisade tissue, and thick cell walls and cuticles. Moreover, antioxidant molecules may play a crucial role in protection against oxidative stress, maintaining the cell redox equilibrium during desiccation. In different species of the genus *Xerophyta*, different magnitudes of some histo-anatomical traits correlated with their mechanism of desiccation tolerance (Lüttge et al., 2011). Desiccation postponement is typical of drought-tolerant plants and includes changes in osmotic adjustment, stomatal closure (in isohydric species), and reduction in leaf expansion with wilting or rolling, among other adaptations. Drought has a profound effect on photosynthesis, especially in isohydric species that regulate stomatal closure with water availability. Stomatal closure leads to suboptimal CO_2 assimilation rates, so light absorption exceeds photosynthetic demand. If excess excitation energy is not avoided it may result in the formation of ROS (Takahashi and Badger, 2011). In turn, if this ROS increase is not counterbalanced by antioxidant defences, it can lead to photo-oxidative damage (Apel and Hirt, 2004). Physiological plasticity in response to different levels of oxidative stress include the increase of different antioxidants and photoprotective molecules such as carotenoids (including xanthophylls), tocopherols, glutathione, ascorbic acid, superoxide dismutase, catalases and peroxidases, among other strategies. Increased levels of the total pool of the xanthophyll cycle components (VAZ: violaxanthin + antheraxanthin + zeaxanthin) and their de-epoxidation state respond to different environmental stresses, as they allow energy dissipation through nonphotochemical quenching (Demmig-Adams et al., 2012). Antioxidants are classified as nonenzymatic (tocopherols, carotenoids, glutathione, ascorbic acid) and enzymatic (catalases, peroxidases and superoxide dismutase,

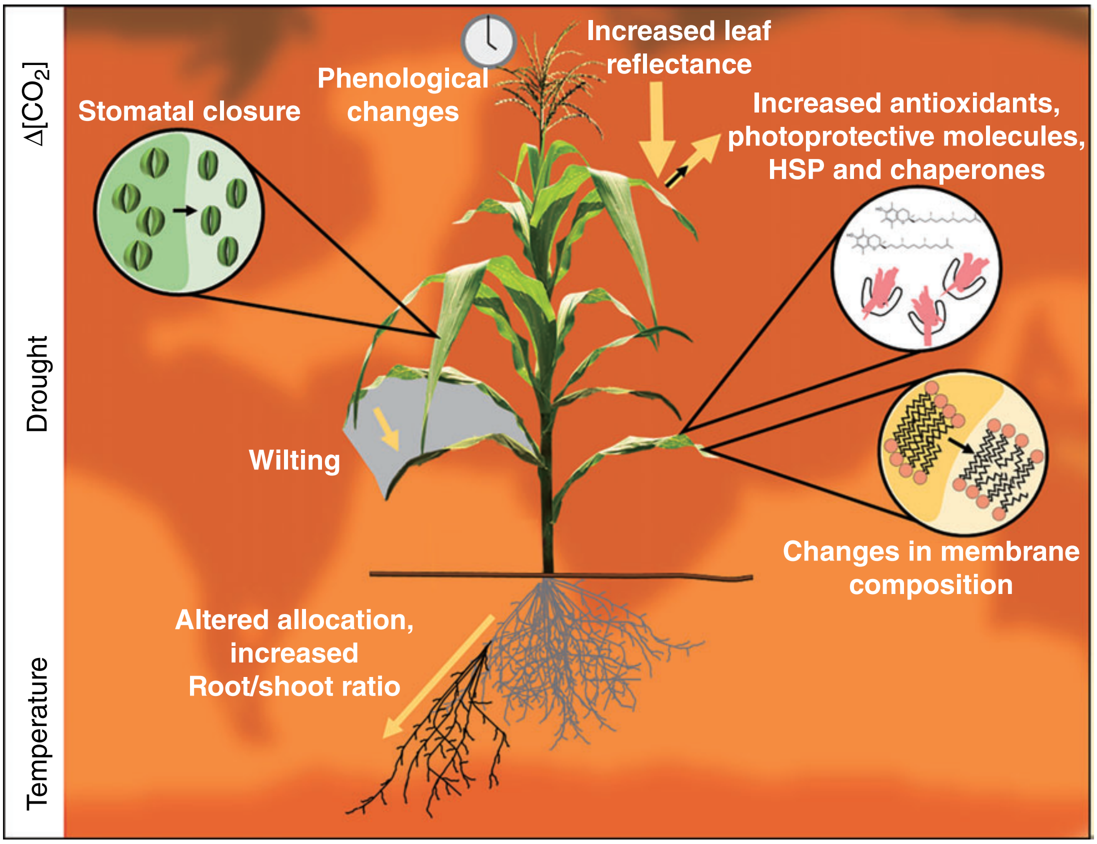

Figure 4 Examples of plant physiological plasticity to main climate change drivers.

among others) and both respond to alterations in the redox cellular equilibrium. Plant responses to low water availability may depend on the severity and duration of the drought period. It has been described that mild drought can increase the water use efficiency through reduced transpiration for a given photosynthetic rate if the stomata are not completely closed. Moreover, relatively mild drought is generally thought to increase partitioning to roots to the detriment of aboveground organs (Blessing et al., 2015). Plant species that exhibit crassulacean acid metabolism (CAM plants) have the highest water use efficiency due to their temporal adaptations to arid environments, having a high plasticity in response to different water availability regimes (Borland et al., 2011, see Figure 4).

6.2 Responses to Unpredictability and Complexity

There are different constraints in predicting plant responses to climate change, mainly due to uncertainty and complexity. Environmental heterogeneity is an important source of complexity as it can be either special or temporal, and it may be not only due to a single environmental condition. The importance of phenotypic plasticity depends on the time course, dynamics, and combinations of climate change-associated factors (Burggren,

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2018). Plants must face multiple global change drivers simultaneously. The increased atmospheric CO_2 levels, higher temperatures, and altered water regimes are not predicted to occur as isolated events but simultaneously. For example, fewer and more closed stomata under high CO_2 levels decrease heat tolerance in plants, especially under low water availability. Moreover, other traits can be contrarily affected by different drivers. Water use efficiency may increase under high CO_2 but decrease under elevated temperature. Conjugation of stresses and environmental cues can lead to neutrality, synergy, antagonism, idiosyncrasy, or dominance (Coté et al., 2016). For example, elevated CO_2 attenuated the negative effects of low water availability in *Solanum lycopersicum* (Wei et al., 2018). Xylem vessel size decreased in the dwarf shrub *Vaccinium myrtillus* in a nine years FACE study in Switzerland (Anadon-Rosell et al., 2018). However, after six years of soil warming (+4 °C) vessel size was more sensitive to temperature than CO_2, showing a significant increase. In other cases, it has been shown that hardening in response to a stressful factor can bring resistance to other stresses.

Another source of complexity are the interactions between different species. Advancement or delay of some life events for some species in response to a temperature rise may have effects at the ecosystem level due to the interaction with other species (Memmott et al., 2007). A three-growing season experiment with solitary bees and wasps at sites along an elevation gradient in the Rocky Mountains revealed that plants are more likely than insects to advance phenology in response to springtime warming (Forrest and Thomson, 2011). Because species have different plasticity, global drivers may have impacts on community assembly. Niche availability and species competitive ability may determine the evolution of plant communities under global change drivers (Valladares et al., 2015). Plasticity of dominant or engineer species in an ecosystem (or ecosystem plasticity) determines the capacity of a particular space to maintain its shape and function. More research towards the understanding of ecosystem plasticity must be addressed to unravel the impacts of climate change.

Male and female individuals from dioecious plants such as *Mercularis annua* showed contrasting phenotypic plasticity in response to climate change (Tonnabel et al., 2017). Moreover, plasticity in sex ratio has been observed for *Silene littorea* in response to different environmental conditions, which adds more complexity when modelling plants depending on their sexual strategy and its plasticity (Buide et al., 2018). Genetic heterogeneity among the territory may also lead to increased difficulties to forecast species impacts under climate change conditions. Individuals from different populations may differ in phenotypic characters, genetic structure, and distinct phenotypic responses to environmental conditions (Sultan and Spencer, 2002). Moreover, environmental sensitivity may be different among the species distribution range, as exposed in the model by Chevin et al. (2010). In this respect, Droste et al. (2010) also show different plasticity

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among populations of one species and that gene flow plays a major role in facilitating adaptation to climate change by admixture between genetically differentiated populations.

7 How Do We Evaluate Plasticity?

Diversity in plant stress responses has been largely studied. However, obtaining measures of phenotypic plasticity may not always be as easy as obtaining measures of other functional traits. As plasticity depends on the environmental conditions, nonreversible responses may cause an overestimation of the plasticity. Moreover, some traits may be more plastic than others and genotype effects may confuse plastic changes. Furthermore, the adaptive nature of plasticity is difficult to test because the plasticity itself prevents the expression of inappropriate phenotypes in any given environment. The measurement of fitness is also not standard and not only short-term fitness may be influenced by plastic responses. All these factors increase the complexity of studying phenotypic plasticity. Here, we present a summary of the used approaches, experimental designs, and statistical techniques to assess phenotypic plasticity in plants.

7.1 Approaches and Experimental Designs

Approaches to phenotypic plasticity may depend on the final aim of the study. We summarise different approaches and their advantages and limitations in Table 1. From a general point of view, studies can be performed at different locations: under controlled conditions that can easily be modified, semi-natural or common garden experiments that allow reciprocal transplant experiments, and under natural conditions. Laboratory or common-garden studies determine whether observed differences among field populations have a genetic basis, whereas reciprocal transplant experiments show whether organisms are most fit in their sites of origin (Parker et al., 2003). Studies under natural conditions allow the analysis of real results of multiple stress factors and plasticity can be seen after a natural gradient in time or space.

If the aim is to contrast plastic *vs.* fixed genotypes, one approach is to perform resurrection studies, based on the comparison of contemporary reaction norms in ancestral *vs.* derived population sets, as well as reciprocal transplant experiments between zones with high and low climatic stochasticity (Ghalambor et al., 2007; Franks et al., 2013). The use of recombinant inbred lines (RILs) is a promising field to understand the genetic basis of physiological plasticity, such as the case of studies with an *A. thaliana* population polymorphic at FRIGIDA (FRI) locus (Callahan

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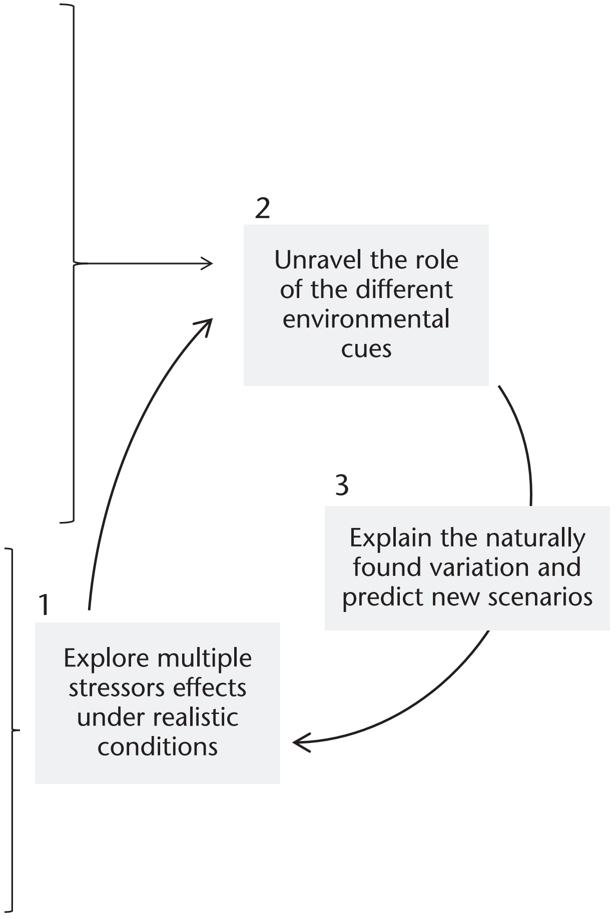
Table 1 Strengths and limitations of different approaches to study phenotypic plasticity.

Experimental design		Strengths		Limitations
		Common	Particular	
Controlled conditions	Mutants	Environmental stressor and clonal individuals can be used	Plastic genes can be identified	Not naturally evolved organisms may be used
	Resurrection studies		Comparable viable species are used	Not available for all species
	Other species		Environmental stressor is isolable	Multiple stressors are not considered
Semi-natural conditions	Mutants	Multiple stressors consideration with the possibility to some environmental control. Clonal individuals can be used	Plastic genes can be identified	Not naturally evolved organisms may be used
	Resurrection studies		Comparable viable species are used	Not available for all species
	Reciprocal transplant		GxE interactions are identified	Conditions may not be representative to the naturally occurring ones
	One species		Multiple stressors are considered	Conditions may not be representative to the naturally occurring ones
Natural conditions	Time gradient	Multiple stressors consideration under realistic environment	Maintenance of the same individuals	Time consuming. Environmental variation may be limited
	Space gradient		Fast in time. Spatial gradient may respond to higher environmental variation in comparison to time	Depends on species actual distribution. It does not study the same genotype
	Dendroecology		Long term past study	Plastic responses are restricted to a few indicators



et al., 2005). Ideally, because plasticity in a trait is defined as the property of a genotype, experiments should be conducted using clonal replicates and not different individuals, which restricts the studies to controlled or common garden studies (Table 1). However, Gianoli and Valladares (2012) pointed out that for certain study aims, the use of genetically identical individuals is not as important in studies of natural populations and natural diversity.

Manipulative experiments under controlled conditions allows us to isolate environmental cues and examine the plant response to different variable intensities and regimes. Manipulative experiments under natural conditions exist (e.g. OTC) but the identification of the drivers of plastic changes is more difficult. Environmental gradients under natural conditions can be explored to evaluate plastic changes of natural populations, including both time and space gradients. Under natural conditions, we cannot properly contrast genotypes that do not occupy the same geographic areas. Reciprocal transplant experiments ensure the GxE effect under semi-natural conditions. The appropriated examination of plant phenotypic responses to assess future performance under climate change conditions requires deep studies considering together the exploration of multiple stressors under natural conditions and manipulative controlled studies to identify the environmental cues responsible for the plastic changes (Table 1).

7.2 The Measurement of Plastic Traits and Fitness

The selection of traits to evaluate plastic responses is not always easy. Some traits are fixed, and some others are extremely plastic to certain environmental changes and for some species. Moreover, the selection of the time of the day or the selected tissue may also influence our results. For example, *S. littorea* has different flavonoid content plasticity in petals and leaves (Del Valle et al., 2018). Some traits may have adaptive plasticity whereas some others may not. The identification of trait elasticity to fitness is essential, but hard in practice. Indeed, the measure of fitness itself may be difficult, as different estimators can be taken, and at different time scales (as shown in Figure 1).

Once we have detected the existence of a plastic response, its evaluation is the next challenge. Valladares et al. (2006) summarised different methods to measure plasticity, but some aspects must be considered. The most used indexes are measures of the plastic trait spread such as variance or the coefficient of variation. Sometimes the absolute trait difference between two environments is used. The plasticity index (PI) is the most used index and considers the absolute trait difference standardised by the initial value (Valladares et al., 2006). Other indexes correct the last value by the environmental difference between the two evaluated points, such as the environmentally standardised plasticity index (ESPI) (Valladares

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et al., 2006). Slope, intercept, or higher-order curvature are also commonly used indexes of phenotypic plasticity after fitting reaction norm functions.

All those indexes consider only a single trait once and therefore are not really defining the individual phenotypic plasticity (Forsman, 2015). Other indexes have been proposed to fully integrate the whole phenotype based on Euclidian distance and multivariate analysis (Chun, 2011; Fenollosa et al., 2017). Other new methods to evaluate plasticity are structural equation models (SEM) that quantify traits trade-offs (Godoy and Valladares, 2012). Despite all methods being capable of ranking genotypes in terms of greater or lesser magnitude of plasticity, there is no universal index for phenotypic plasticity comparison yet (Callahan et al., 2008).

8 Summary Points

Phenotypic plasticity is considered to be crucial on the survival and adaptation of organisms to changing environmental conditions. However, the adaptive role of plastic responses may depend on several aspects such as environmental pressure, term of the evaluation or frequency of environmental exposure, and the classification of plastic responses into adaptive and nonadaptive may be somewhat inaccurate. Stress promotes the expression of plastic changes that need to be carefully analysed, and recovery capacity may be crucial in determining the benefits of plasticity. Stress memory needs to be considered when understanding plant adaptation to climate change. Limits and costs of plasticity exist and may alter species capacity to adapt to new environments. Plastic development compromises species further success and can prevent maladaptive phenotypes. New genetic tools allow a better assessment of phenotypic plasticity, with the identification of the genetic architecture behind plastic responses and their heritability.

Plant responses to increased CO_2, temperature, and alterations in water regimes may lead to different and contradictory plastic changes, and therefore it is necessary to use a combined mechanistic approach to elucidate the magnitude and direction of the future changes considering together all the climate change drivers. Phenological changes, altered stomatal conductance, morphological leaf alterations, changes in membrane composition and increased contents of antioxidants and protective molecules are some of the predicted plastic changes of plants facing climate change. More studies are needed to fully elucidate the role of phenotypic plasticity in climate change adaptation. New experimental approaches must consider both manipulative and realistic experiments and an integrated quantification of phenotypic plasticity and fitness.

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References

Aljazairi, S. and Arias, C. (2015). Carbon and nitrogen allocation and partitioning in traditional and modern wheat genotypes under pre-industrial and future CO_2 conditions. *Plant Biology* **17**: 647–659.

Amthor, J.S. (2001). Effects of atmospheric CO_2 concentration on wheat yield : review of results from experiments using various approaches to control CO_2 concentration. *Field Crops Research* **73**: 1–35.

Anadon-Rosell, A., Dawes, M.A., Fonti, P. et al. (2018). Xylem anatomical and growth responses of the dwarf shrub *Vaccinium myrtillus* to experimental CO_2 enrichment and soil warming at treeline. *Science of the Total Environment* **642**: 1172–1183.

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55**: 373–399.

Asseng, S. (2015). Rising temperatures reduce global wheat production. *Nature Climate Change* **5**: 143–147.

Auge, G.A., Blair, L.K., Karediya, A., and Donohue, K. (2018). The autonomous flowering-time pathway pleiotropically regulates seed germination in *Arabidopsis thaliana*. *Annals of Botany* **121**: 183–191.

Avolio, M.L., Hoffman, A.M., and Smith, M.D. (2018). Linking gene regulation, physiology, and plant biomass allocation in *Andropogon gerardii* in response to drought. *Plant Ecology* **219**: 1–15.

Balao, F. (2018). Uncovering the contribution of epigenetics to plant phenotypic variation in Mediterranean ecosystems. *Plant Biology* **20**: 38–49.

Beaman, J.E., White, C.R., and Seebacher, F. (2016). Evolution of plasticity: mechanistic link between development and reversible acclimation. *Trends in Ecology & Evolution* **31**: 237–249.

Bigot, S., Buges, J., Gilly, L. et al. (2018). Pivotal roles of environmental sensing and signaling mechanisms in plant responses to climate change. *Global Change Biology* **24** (**12**): 5573–5589.

Annual Plant Reviews Online, Volume 2. Edited by Jeremy Roberts.

Blessing, C.H., Werner, R.A., Siegwolf, R., and Buchmann, N. (2015). Allocation dynamics of recently fixed carbon in beech saplings in response to increased temperatures and drought. *Tree Physiology* **35**: 585–598.

Borland, A.M., Zambrano, V.A.B., Ceusters, J., and Shorrock, K. (2011). The photosynthetic plasticity of crassulacean acid metabolism: an evolutionary innovation for sustainable productivity in a changing world. *New Phytologist* **191**: 619–633.

Bossdorf, O., Richards, C.L., and Pigliucci, M. (2008). Epigenetics for ecologists. *Ecology Letters* **11**: 106–115.

Brumos, J., Robles, L.M., Yun, J. et al. (2018). Local auxin biosynthesis is a key regulator of plant article local auxin biosynthesis is a key regulator of plant development. *Developmental Cell* **47**: 306–318.e5.

Buide, M.L., del Valle, J.C., Castilla, A.R., and Narbona, E. (2018). Sex expression variation in response to shade in gynodioecious-gynomonoecious species: *Silene littorea* decreases flower production and increases female flower proportion. *Environmental and Experimental Botany* **146**: 54–61.

Burggren, W. (2018). Developmental phenotypic plasticity helps bridge stochastic weather events associated with climate change. *Journal of Experimental Biology* **221**: 1–9.

Callahan, H.S., Dhanoolal, N., Ungerer, M.C., and Callahan, H.S. (2005). Plasticity genes and plasticity costs: a new approach using an Arabidopsis recombinant inbred population. *New Phytologist* **166**: 129–140.

Callahan, H.S., Maughan, H., and Steiner, U.K. (2008). Phenotypic plasticity, costs of phenotypes, and costs of plasticity toward an integrative view. *Annals of the New York Academy of Sciences* **1133**: 44–66.

Catoni, R., Gratani, L., and Varone, L. (2012). Physiological, morphological and anatomical trait variations between winter and summer leaves of *Cistus* species. *Flora* **207**: 442–449.

Chevin, L., Lande, R., and Mace, G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology* **8** (**4**): e1000357.

Choi, W., Toyota, M., Kim, S. et al. (2014). Salt stress-induced Ca^{2+} waves are associated with rapid, long-distance root-to-shoot signaling in plants. *PNAS* **111**: 6497–6502.

Chun, Y.J. (2011). Phenotypic plasticity of introduced versus native purple loosestrife: univariate and multivariate reaction norm approaches. *Biological Invasions* **13**: 819–829.

Colautti, R.I. and Barrett, S.C.H. (2013). Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science* **342**: 364–366.

Coté, I.M., Darling, E.S., and Brown, C.J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B* **283**: 1–9.

Crisp, P.A., Ganguly, D., Eichten, S.R. et al. (2016). Reconsidering plant memory: intersections between stress recovery, RNA turnover, and epigenetics. *Science Advances* **2** (**2**): e1501340.

Dalal, A., Attia, Z., and Moshelion, M. (2017). To produce or to survive: how plastic is your crop stress physiology? *Frontiers in Plant Science* **8**: 1–8.

Del Valle, C.J., Buide, M.L., Whittall, J.B., and Narbona, E. (2018). Phenotypic plasticity in light-induced flavonoids varies among tissues in *Silene littorea* (Caryophyllaceae). *Environmental and Experimental Botany* **153**: 100–107.

Annual Plant Reviews Online, Volume 2. Edited by Jeremy Roberts.

Demmig-Adams, B., Cohu, C.M., Muller, O., and William, W.A. (2012). Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynthesis Research* **113**: 75–88.

Dodd, M.S., Papineau, D., Grenne, T. et al. (2017). Evidence for early life in Earth's oldest hydrothermal vent precipitates. *Nature* **543**: 60–64.

Dorn, L.A., Pyle, E.H., and Schmitt, J. (2000). Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution* **54**: 1982–1994.

Droste, T., Flory, S.L., and Clay, K. (2010). Variation for phenotypic plasticity among populations of an invasive exotic grass. *Plant Ecology* **207**: 297–306.

Du, C., Wang, X., Zhang, M. et al. (2019). Effects of elevated CO_2 on plant C-N-P stoichiometry in terrestrial ecosystems: a meta-analysis. *Science of the Total Environment* **650**: 697–708.

Edelaar, P., Jovani, R., and Gomez-Mestre, I. (2017). Should I change or should I go? Phenotypic plasticity and matching habitat choice in the adaptation to environmental heterogeneity. *The American Naturalist* **190**: 506–520.

Ernande, B. and Dieckmann, U. (2004). The evolution of phenotypic plasticity in spatially structured environments: implications of intraspecific competition, plasticity costs and environmental characteristics. *Journal of Evolutionary Biology* **17**: 613–628.

Fenollosa, E., Munné-bosch, S., and Pintó-marijuan, M. (2017). Contrasting phenotypic plasticity in the photoprotective strategies of the invasive species *Carpobrotus edulis* and the coexisting native species *Crithmum maritimum*. *Physiologia Plantarum* **160**: 185–200.

Fenollosa, E., Gámez, A., and Munné-bosch, S. (2018). Plasticity in the hormonal response to cold stress in the invasive plant *Carpobrotus edulis*. *Journal of Plant Physiology* **231**: 202–209.

Fischer, E.M., Beyerle, U., Schleussener, C.F. et al. (2018). Biased estimates of changes in climate extremes from prescribed SST simulations. *Geophysical Research Letters* **45**: 8500–8509.

Forrest, J.R.K. and Thomson, J.D. (2011). An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs* **81**: 469–491.

Forsman, A. (2015). Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* **115**: 276–284.

Franks, S.J., Weber, J.J., and Aitken, S.N. (2013). Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications* **7**: 123–139.

Gabriel, W. (2005). How stress selects for reversible phenotypic plasticity. *Journal of Evolutionary Biology* **18**: 873–883.

Gagliano, M., Renton, M., Depczynski, M., and Mancuso, S. (2014). Experience teaches plants to learn faster and forget slower in environments where it matters. *Behavioral Ecology* **175**: 63–72.

Ghalambor, C.K., McKay, J.K., Carroll, S.P., and Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new. *Functional Ecology* **21**: 394–407.

Gianoli, E. and Palacio-López, K. (2009). Phenotypic integration may constrain phenotypic plasticity in plants. *Oikos* **118**: 1924–1928.

Gianoli, E. and Valladares, F. (2012). Studying phenotypic plasticity: the advantages of a broad approach. *Biological Journal of the Linnean Society* **105**: 1–7.

Annual Plant Reviews Online, Volume 2. Edited by Jeremy Roberts.

Godoy, O. and Valladares, F. (2012). The relative importance for plant invasiveness of trait means, and their plasticity and integration in a multivariate framework. *New Phytologist* **195**: 912–922.

Hedrich, R., Salvador-recatalà, V., and Dreyer, I. (2016). Electrical wiring and long-distance plant communication. *Trends in Plant Science* **21**: 376–387.

Hendrey, G.R., Ellsworth, D.S., Lewin, K.F., and Nagy, J. (1999). A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology* **5**: 293–309.

Herman, J.J. and Sultan, S.E. (2011). Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science* **2**: 1–10.

Hughes, T.P., Kerry, J.T., Connolly, S.R. et al. (2019). Ecological memory modifies the cumulative impact of recurrent climate extremes. *Nature Climate Change* **9**: 40–43.

Jung, J., Domijan, M., Klose, C. et al. (2016). Phytochromes function as thermosensors in Arabidopsis. *Science* **354**: 886–889.

Knight, T.M., Steets, J.A., Vamosi, J.C. et al. (2005). Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* **36**: 467–497.

Koini, M.A., Alvey, L., Allen, T. et al. (2009). High temperature-mediated adaptations in plant architecture require the bHLH transcription Factor PIF4. *Current Biology* **19**: 408–413.

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology* **22**: 1435–1446.

Latta, L.C., Weider, L.J., Colbourne, J.K., and Pfrender, M.E. (2012). The evolution of salinity tolerance in Daphnia: a functional genomics approach. *Ecology Letters* **15**: 794–802.

Legris, M., Klose, C., Burgie, E.S. et al. (2016). Phytochrome B integrates light and temperature signals in Arabidopsis. *Science* **354**: 897–900.

Long, S.P., Ainsworth, E.A., Rogers, A., and Ort, D.R. (2004). Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55**: 591–628.

Lüttge, U., Beck, E., and Bartels, D. (2011). *Plant Desiccation Tolerance*. Springer-Verlag.

Marchiori, P.E.R., Machado, E.C., Sales, C.R.G. et al. (2017). Physiological plasticity is important for maintaining sugarcane growth under water deficit. *Frontiers in Plant Science* **8**: 1–12.

Mathur, S. and Jajoo, A. (2014). Alterations in photochemical efficiency of photosystem II in wheat plant on hot summer day. *Physiology and Molecular Biology of Plants* **20**: 527–531.

Memmott, J., Craze, P.G., Waser, M., and Price, M.V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecology Letters* **10**: 710–717.

Menzel, A., Sparks, T.H., Estrella, N. et al. (2006). European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**: 1969–1976.

Merilä, J. and Hendry, A.P. (2013). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications* **7**: 1–14.

Michmizos, D. and Hilioti, Z. (2019). A roadmap towards a functional paradigm for learning and memory in plants. *Journal of Plant Physiology* **232**: 209–215.

Annual Plant Reviews Online, Volume 2. Edited by Jeremy Roberts.

Mikolajewski, D.J., Johansson, F., Wohlfahrt, B., and Stoks, R. (2006). Invertebrate predation selected for the loss of a morphological antipredator trait. *Evolution* **60**: 1306–1310.

Miller, G., Schlauch, K., Tam, R. et al. (2009). The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling* **2**: ra45.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science* **11**: 1360–1385.

Munné-Bosch, S., Queval, G., and Foyer, C.H. (2013). The impact of global change factors on redox signaling underpinning stress tolerance. *Plant Physiology* **161**: 5–19.

Murren, C.J., Auld, J.R., Callahan, H. et al. (2015). Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity* **115**: 293–301.

Nakashima, S., Kebukawa, Y., Kitadai, N., and Igisu, M. (2018). Geochemistry and the origin of life: from extraterrestrial processes, chemical evolution on earth, fossilized life's records, to natures of the extant life. *Life (Basel, Switzerland)* **8**: 1–21.

Nakayama, H., Sinha, N.R., and Kimura, S. (2017). How do plants and phytohormones accomplish heterophylly, leaf phenotypic plasticity, in response to environmental cues. *Frontiers in Plant Science* **8**: 1–7.

Narayanan, S., Tamura, P.J., Roth, M.R. et al. (2016). Wheat leaf lipids during heat stress: high day and night temperatures result in major lipid alterations. *Plant, Cell and Environment* **39**: 787–803.

Nievola, C.C., Carvalho, C.P., Carvalho, V. et al. (2017). Rapid responses of plants to temperature changes. *Temperature* **4**: 371–405.

Parker, I.M., Rodriguez, J., and Loik, M.E. (2003). An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conservation Biology* **17**: 59–72.

Pei, Z., Murata, Y., Benning, G.J. et al. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **406**: 731–734.

Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution* **20**: 481–486.

de Pinto, M.C., Locato, V., Paradiso, A., and De Gara, L. (2015). Role of redox homeostasis in thermo-tolerance under a climate change scenario. *Annals of Botany* **116**: 487–496.

Price, T.D., Qvarnström, A., and Irwin, D.E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences* **270**: 1433–1440.

Puglielli, G., Gratani, L., Catoni, R. et al. (2017). Short-term physiological plasticity: trade-off between drought and recovery responses in three Mediterranean *Cistus* species. *Ecology and Evolution* **7**: 10880–10889.

Ramel, F., Birtic, S., Ginies, C. et al. (2012). Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *PNAS* **109**: 5535–5540.

Raschke, K., Shabahang, M., and Wolf, R. (2003). The slow and the quick anion conductance in whole guard cells: their voltage-dependent alternation, and the modulation of their activities by abscisic acid and CO_2. *Planta* **217**: 639–650.

Reich, P.B., Hobbie, S.E., Lee, T.D., and Pastore, M.A. (2018). Unexpected reversal of C_3 versus C_4 grass response to elevated CO_2 during a 20-year field experiment. *Science* **360**: 317–320.

Annual Plant Reviews Online, Volume 2. Edited by Jeremy Roberts.

Rentel, M.C., Knight, M.R., and Kingdom, U. (2004). Oxidative stress-induced calcium signaling. *Plant Physiology* **135**: 1471–1479.

Rezaei, E.E. (2018). Climate change effect on wheat phenology depends on cultivar change. *Scientific Reports* **8**: 1–10.

Richards, E.J. (2006). Inherited epigenetic variation: revisiting soft inheritance. *Nature Reviews* **7**: 395–402.

Rico, L., Ogaya, R., and Barbeta, A. (2014). Changes in DNA methylation fingerprint of *Quercus ilex* trees in response to experimental field drought simulating projected climate change. *Plant Biology* **16**: 419–427.

Rubio de Casas, R., Vargas, P., Perez-Corona, E. et al. (2009). Variation in sclerophylly among Iberian populations of *Quercus coccifera* L. is associated with genetic differentiation across contrasting environments. *Plant Biology* **11**: 464–472.

Saidi, Y., Peter, M., Finka, A. et al. (2010). Membrane lipid composition affects plant heat sensing and modulates Ca^{2+}-dependent heat shock response. *Plant Signalling and Behaviour* **5**: 1530–1533.

Scheiner, S.M. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24**: 35–68.

Scheiner, S.M. and Holt, R.D. (2012). The genetics of phenotypic plasticity. X. Variation versus uncertainty. *Ecology and Evolution* **2**: 751–767.

Scheiner, S.M., Barfield, M., and Holt, R.D. (2017). The genetics of phenotypic plasticity. XV. Genetic assimilation, the Baldwin effect, and evolutionary rescue. *Ecology* **7**: 8788–8803.

Seal, C.E., Daws, M.I., Flores, J. et al. (2017). Thermal buffering capacity of the germination phenotype across the environmental envelope of the Cactaceae. *Global Change Biology* **23**: 5309–5317.

Sevanto, S. and Dickman, L.T. (2015). Where does the carbon go?—Plant carbon allocation under climate change. *Tree Physiology* **35**: 581–584.

Sheffield, J., Wood, E.F., and Roderick, M.L. (2012). Little change in global drought over the past 60 years. *Nature* **491**: 435–438.

Snell-rood, E.C. and Carolina, S. (2012). Integrative and comparative biology selective processes in development: implications for the costs and benefits of phenotypic plasticity. *Integrative and Comparative Biology* **52**: 31–42.

Sultan, S.E. and Spencer, H.G. (2002). Metapopulation structure factors plasticity over local adaptation. *The American Naturalist* **160**: 271–283.

Sultan, S.E., Barton, K., and Wilczek, A.M. (2009). Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology* **90**: 1831–1839.

Takahashi, S. and Badger, M.R. (2011). Photoprotection in plants: a new light on photosystem II damage. *Trends in Plant Science* **16**: 53–60.

Tonnabel, J., David, P., and Pannell, J.R. (2017). Sex-specific strategies of resource allocation in response to competition for light in a dioecious plant. *Oecologia* **185**: 675–686.

Tuba, Z. (2008). Notes on the poikilochlorophyllous desiccation-tolerant plants. *Acta Biologica Szegediensis* **52**: 111–113.

Turcotte, M.M. and Levine, J.M. (2016). Phenotypic plasticity and species coexistence. *Trends in Ecology & Evolution* **31**: 803–813.

Valladares, F., Sanchez-gomez, D., and Zavala, M.A. (2006). Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* **94**: 1103–1116.

Annual Plant Reviews Online, Volume 2. Edited by Jeremy Roberts.

Valladares, F., Gianoli, E., and Gómez, J.M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytologist* **176**: 749–763.

Valladares, F., Bastias, C.C., Godoy, O. et al. (2015). Species coexistence in a changing world. *Frontiers in Plant Science* **6**: 1–16.

Van Heerwaarden, B. and Sgrò, C.M. (2017). The quantitative genetic basis of clinal divergence in phenotypic plasticity. *Evolution* **71**: 2618–2633.

Van Kleunen, M. and Fischer, M. (2004). Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* **166**: 49–60.

Van Tienderen, P.H. (1991). Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* **45**: 1317–1331.

Verma, V., Ravindran, P., and Kumar, P.P. (2016). Plant hormone-mediated regulation of stress responses. *BMC Plant Biology* **16**: 86.

Virdi, K.S., Laurie, J.D., Xu, Y. et al. (2015). Arabidopsis MSH1 mutation alters the epigenome and produces heritable changes in plant growth. *Nature Communications* **6**: 1–9.

Wang, Z., Pang, X., and Lv, Y. (2012). A dynamic framework for quantifying the genetic architecture of phenotypic plasticity. *Briefings in Bioinformatics* **14**: 82–95.

Wei, Z., Du, T., Li, X. et al. (2018). Interactive effects of elevated CO_2 and N fertilization on yield and quality of tomato grown under reduced irrigation regimes. *Frontiers in Plant Science* **9**: 1–10.

West-Eberhard, M.J. (2005). Developmental plasticity and the origin of species differences. *PNAS* **102**: 6543–6549.

Woodward, F.I. (2002). Potential impacts of global elevated CO_2 concentrations on plants. *Current Opinion in Plant Biology* **5**: 207–211.

Woodward, F.I., Lake, J.A., and Quick, W.P. (2002). Stomatal development and CO_2: ecological consequences. *New Phytologist* **153**: 477–484.

Zhao, X., Zhou, N., Lai, S. et al. (2019). Elevated CO_2 improves lodging resistance of rice by changing physicochemical properties of the basal internodes. *Science of the Total Environment* **647**: 223–231.

Annual Plant Reviews Online, Volume 2. Edited by Jeremy Roberts.